UNIVERSIDAD DE SANTIAGO DE COMPOSTELA

FACULTAD DE QUÍMICA

Departamento de Química Analítica, Nutrición y Bromatología

Laboratorio de Investigación y Desarrollo de Soluciones Analíticas (LIDSA)

NUEVOS PROCEDIMIENTOS DE PREPARACIÓN DE MUESTRA Y ANÁLISIS CROMATOGRÁFICO PARA LA DETERMINACIÓN DE PRODUCTOS DE CUIDADO PERSONAL Y CONTAMINANTES DE INTERÉS PRIORITARIO

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Memoria para optar al grado de Doctora en Ciencia y Tecnología Química

Santiago de Compostela, 2015

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As Profesoras Doutoras Dna. María Llompart Vizoso e Dna. Carmen García Jares,

como Directoras da tese titulada: ***"Nuevos procedimientos de preparación de muestra y análisis cromatográfico para la determinación de productos de cuidado personal y contaminantes de interés prioritario"***

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E que está en coñecemento dos coautores, tanto doutores como non doutores, participantes nos artigos que ningún dos traballos reunidos nesta tese serán presentados por ningún deles noutra tese de Doutoramento, o que asino baixo a miña responsabilidade.

Santiago de Compostela, a 8 de abril de 2015.

Fdo: María Llompart Vizoso

Fdo: Carmen García Jares

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AGRADECIMIENTOS

Llegados a este punto, echo la vista atrás, y resulta difícil resumir en una sola hoja todos los nombres de los que han formado parte de mi vida durante esta etapa, y que han contribuido en mayor o menor medida a esta Tesis.

En primer lugar me gustaría agradecer a mis directoras María Llompart y Carmen G. Jares por confiar en mí y darme la gran oportunidad de realizar este trabajo con ellas. Por sus ideas y su apoyo en todo momento. Gracias.

A Marta Lores, por sus ánimos y su energía contagiosa y a Pablo por su infinita paciencia conmigo y su ayuda incondicional siempre, desde el primer hasta el último momento.

Al Departamento de Química Analítica, Nutrición y Bromatología por los medios técnicos aportados y a las entidades tanto públicas como privadas que han financiado este trabajo, en especial a la RED Regata por financiar mi estancia de 3 meses en Grecia.

Por supuesto a todos los compañeros que han pasado por el laboratorio en estos años, muy especialmente a los "veteranos" Lucía, Gerardo y Marta A. y a las "peques" Eugenia, Marlene y Marta P. Gracias por los divertidos momentos que hemos pasado.

A la maravillosa gente de la Technical University of Crete, muy especialmente a Elia por su entusiasmo y darme la oportunidad de trabajar con ella y a Elina y a las chicas del Aqua Lab por acogerme y hacerme sentir como una más durante 3 meses. Εχαριτώ.

También agradecer a mis "Tileras" de Lugo por su comprensión, paciencia y ánimo, sobre todo en esta última etapa y como no, a mi gente de Santiago, tanto al "Lawyer Team" como al "Psicoteam" por esos momentos coffee de desconexión tan necesarios, sois geniales!!

Por último, aunque no por ello menos importante, a mis padres y a mi hermano por sus ánimos en los momentos difíciles. Sin su apoyo nunca hubiese podido llegar hasta aquí.

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"La mayor parte de las ideas fundamentales de la ciencia son esencialmente sencillas, y por esa regla general pueden ser expresadas en un lenguaje comprensible para todos"

Albert Einstein

"Sin ambición uno no empieza nada,

sin trabajo uno no termina nada.

Nadie te enviará el premio.

Tendrás que ganarlo"

Ralph Waldo Emerson

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i. Resumen-Summary

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RESUMEN

El objetivo principal de esta Tesis Doctoral es el desarrollo de nueva metodología para el análisis de gran variedad de compuestos químicos de naturaleza muy diversa en distintos tipos de muestras. Los métodos propuestos se basan en técnicas de extracción muy sencillas y rápidas que tratan de cumplir en la medida de lo posible con los principios de "Química Verde", empleando cantidades mínimas de disolventes orgánicos y con una generación de residuos casi nula.

El trabajo se divide en tres Capítulos según los compuestos que se pretenden estudiar. De esta forma, el ***Capítulo I*** se centra en la ***determinación se sustancias potencialmente tóxicas en productos cosméticos y de cuidado personal***. El objetivo era desarrollar un método analítico sencillo para la determinación simultánea de una gran variedad de compuestos de naturaleza química muy diversa y que pudiese ser aplicado a diferentes formas cosméticas. Para ello, se empleó como técnica de extracción una miniaturización de la ***dispersión de matriz en fase sólida*** (*matrix solid-phase dispersion, MSPD*) lo que conlleva una reducción de la cantidad de muestra y de disolventes orgánicos. Esta µ-MSPD se realizó con material de uso común en cualquier laboratorio, por lo que su coste es mínimo. Para el análisis de un producto de cuidado personal específico, como son las toallitas infantiles se empleó la ***extracción con líquidos presurizados*** (*pressurized liquid extraction, PLE*). En ambos casos las condiciones de extracción fueron optimizadas mediante diseños experimentales para obtener la máxima eficacia. La determinación se llevó a cabo mediante ***cromatografía de gases-espectrometría de masas*** (***GC-MS)*** trabajando tanto en modo simple (MS) como en tándem (MS/MS), lo que permitió obtener una mayor selectividad y, por lo tanto, sensibilidad analítica. De esta forma, se ha obtenido una metodología analítica fiable y robusta, validada en términos de linealidad, exactitud y precisión, capaz de determinar casi 70 compuestos de muy diversa naturaleza química.

El ***Capítulo II*** se centra en el desarrollo de métodos para el ***análisis de fungicidas*** tanto en ***vino*** como en ***sub-productos de vinificación***. En estos casos, como técnicas de extracción se emplearon la ***microextracción-emulsificación asistida por ultrasonidos*** (*ultrasound-assisted emulsification-microextraction, USAEME*) para el análisis de estos compuestos en vino y, tanto ***PLE*** como la ***extracción asistida por ultrasonidos*** (*ultrasound assisted extraction, UAE*), para el análisis de bagazo de uva blanca. Al igual que en el caso anterior, se empleó GC-MS y GC-MS/MS, obteniéndose en ambos casos, métodos adecuados para el objetivo propuesto.

El ***Capítulo III*** se dedica al ***estudio de los hidrocarburos aromáticos policíclicos*** (PAHs) en diversos tipos de muestras. En este sentido, tanto ***UAE*** como la ***microextracción en fase sólida*** (*solid-phase microextraction, SPME*) seguidas de GC-MS, se utilizaron con éxito para demostrar la presencia de estos contaminantes prioritarios en ***superficies de caucho reciclado***, empleadas como suelos de parques infantiles, así como en el aire y agua que se encuentran en contacto con las mismas. Por último, en este Capítulo se incluye la optimización de las condiciones experimentales de SPME con vacío (***Vac-SPME***) con el objetivo de obtener la mayor eficacia de extracción, para que esta técnica pueda ser aplicada al análisis de ***aceite de oliva***.

SUMMARY

The main objective of this PhD Thesis is the development of new analytical methods to determine several chemical compounds in different samples. The proposed methodology is based on simple and fast extraction techniques; they were developed with the aim to fulfil with the 'Green Chemistry Principles', minimizing or even avoiding solvent consumption and the generation of waste.

This work is divided in 3 chapters according to the studied compounds. In this way, ***Chapter I*** is focused on the ***determination of hazardous substances in cosmetics and personal care products***. The objective was to develop a simple analytical method for the simultaneous analysis of several chemical forms in a broad range of cosmetics. A low-cost extraction technique based on *matrix solid-phase dispersion, MSPD*, was employed and miniaturized to reduce risks of contamination, residues and costs. For the analysis of a specific personal care product intended for newborns and babies, *pressurized liquid extraction, PLE*, was successfully employed as extraction technique. In both cases, experimental conditions were optimized by means of experimental designs in order to obtain the maximum efficacy. Determination of target compounds was carried out by gas chromatography-mass spectrometry (GC-MS), working under single (MS) or tandem mode (MS/MS) in order to obtain selectivity and analyte sensitivity. Finally, all the proposed methods were validated in terms of linearity, accuracy and precision.

Chapter II is focused on the ***analysis of fungicides in wine and*** its most important vinification sub-product, ***bagasse***, which is also employed as source of natural bioactive compounds in pharmaceutical or cosmetic applications. *Ultrasound-assisted emulsification-microextraction, USAEME* followed by GC-MS was successfully employed to determine several fungicides in wine, while *PLE* and *ultrasound assisted extraction, UAE* followed by GC-MS/MS were used for the determination of target from the white grape bagasse.

Finally, ***Chapter III*** is dedicated to the ***study of polycyclic aromatic hydrocarbons*** (PAHs) ***in*** different types of samples. In this way, *UAE* and *solid-phase microextraction, SPME* followed by GC-MS were employed to demonstrate the presence of PAHs and other hazardous contaminants in ***recycled tyre rubber surfaces*** employed as playgrounds and to prove a transfer between the surfaces and the air and water put in contact with them. Lastly, this Chapter includes the obtained results during the short stay at the Technical University of Crete, where different experimental conditions of vacuum-SPME were tested in order to obtained the highest extraction efficacy to develop a method for the analysis of PAHs in olive oil.

ii. Abreviaturas y acrónimos

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ABREVIATURAS Y ACRÓNIMOS

A

ACE	Acenapthene	Acenafteno
ACY	Acenapthylene	Acenaftileno
ANC	Anthracene	Antraceno
ANOVA	Analysis of variance	Análisis de varianza
ANSM	French National Agency for the Medicament Security	Agencia Nacional Francesa de Seguridad de Medicamentos
ASE®	Accelerated Solvent Extraction	Extracción acelerada con disolventes

B

B(A)a	Benzo(a)anthracene	Benzo(a)antraceno
B(a)P	Benzo(a)pyrene	Benzo(a)pireno
B(b)F	Benzo(b)fluoranthene	Benzo(a)fluoranteno
BBP	Benzylbutylphthalate	Ftalato de bencilbutilo
BDX	Bronidox	Bronidox
B(gih)P	Benzo(g,h,i)perylene	Benzo(g,h,i)perileno
BHA	Butylhydroxyanisole	Hidroxibutilanisol
BHT	Butylhydroxytoluene	Hidroxibutilo de tolueno
B(k)F	Benzo(k)fluoranthene	Benzo(k)fluoranteno
BuP	Butylparaben	Butilparabeno
BzP	Benzylparaben	Bencilparabeno

C

CAS	Chemical Abstracts Service	
CE	European Commission	Comisión Europea
CHY	Chrysene	Criseno

D

D(ah)A	Dibenz(a,h)anthracene	Dibenz(a,h)antraceno
DBP	Dibutylphthalate	Ftalato de dibutilo
DCHP	Dicyclohexylphthalate	Ftalato de diciclohexilo
DEA	Diethyladipate	Dietiladipato

DEHA	Di-2-(ethylhexyl)adipate	Di-2-etilhexiladipato
DEHP	Di-2(ethylhexyl)phthalate	Ftalato de 2-etilhexilo
DEP	Diethylphthalate	Ftalato de dietilo
DI	Direct injection	Inyección directa
DIBP	Diisobutylphthalate	Ftalato de diisobutil
DIDP	Diisodecylphthalate	Ftalato de diisodecilo
DIHP	Diisoheptylphthalate	Ftalato de diisoheptilo
DINP	Diisononylphthalate	Ftalato de diisononilo
DIPP	Diisopentylphthalate	Ftalato de diisopentilo
DMA	Dimethyladipate	Dimetiladipato
DMP	Dimethylphthalate	Dimethylphthalate
DMEP	Dimethoxyetylphthalate	Ftalato de dimetoxietilo
DNOP	Di-n-octylphthalate	Ftalato de di-n-octilo
DPhP	Diphenylphthalate	Ftalato de difenilo
DIPP	Diisopentylphthalate	Ftalato de diisopentilo
DPP	Dipentylphthalate	Ftalato de dipentilo
DVB	Divinylbenzene	Divinilbenceno

E

EC	European Community	Comunidad Europea
ECHA	European Chemicals Agency	Agencia Europea de sustancias y Mezclas Químicas
EPA	Environmental Protection Agency	Agencia de Protección Medioambiental
EtP	Ethylparaben	Etilparabeno
EU	European Union	Unión Europea

F

FD&C Act	Federal Food, Drug and Cosmetic Act	
FDA	Food and Drug Administration	
FLA	Fluoranthene	Fluoranteno
FLU	Fluorene	Fluoreno

G

g.	Gram	Gramo
GC	Gas chromatography	Cromatografía de gases
GC-MS	Gas chromatography-mass spectrometry	Cromatografía de gases-espectrometría de masas
GC-MS/MS	Gas chromatography-tandem mass spectrometry	Cromatografía de gases-espectrometría de masas en tándem
GHS	Globally harmonized system	Sistema Globalmente Armonizado

H

HPLC	High Performance Liquid Chromatography	Cromatografía líquida de alta resolución
HS	Headspace	Espacio de cabeza
HS-SPME	Headspace-solid-phase microextraction	Microextracción en fase sólida en espacio de cabeza

I

iBuP	Isobutylparaben	Isobutilparabeno
ICCR	Internatinal Cooperation on Cosmetics Regulation	
i.d.	Internal diameter	Diámetro interno
IDL	Instrumental detection limit	Límite de detección instrumental
IND	Indeno[1,2,3-cd]pyrene	Indeno[1,2,3-cd]pireno
IPBC	Iodopropynylbutylcarbamate	Butilcarbamato de iodopropilbutilo
iPrP	Isopropylparaben	Isopropilparabeno

K

Kg	Kilogram	Kilogramo
K_H	Henry's constant	Constante de Henry
K_{OW}	Partition coefficient octanol-water	Coeficiente de partición octanol-agua

L

LC	Liquid chromatography	Cromatografía líquida

LC-MS	Liquid chromatography-mass spectrometry	Cromatografía líquida-espectrometría de masas
LC_{50}	Air letal dose	Dosis letal en aire
LD_{50}	Letal Dose	Dosis letal
LOD	Limit of detection	Límite de detección
LOQ	Limit of quantification	Límite de cuantificación

M

MeP	Methylparaben	Metilparabeno
min	Minute	Minuto
mL	Mililitre	Mililitro
MRL	Maximum Residue Limit	Límite Máximo de Residuos
MS	Mass spectrometry	Espectrometría de masas
MS/MS	Tandem mass spectrometry	Espectrometría de masas en tándem
MSPD	Matrix solid-phase dispersion	Dispersión de matriz en fase sólida
µg	Microgram	Microgramo
µL	Microlitre	Microlitro
µ-MSPD	Micro-matrix solid-phase dispersion	Micro-dispersión de matriz en fase sólida

N

NAP	Naphthalene	Naftaleno
NaCl	Sodium Chloride	Cloruro sódico
Na_2SO_4	Sodium Sulphate	Sulfato sódico

O

OMS	World Health Organization	Organización Mundial de la Salud

P

PAH	Polycyclic aromatic hydrocarbon	Hidrocarburo aromatic policíclico

PCP	Personal care product	Producto de cuidado personal
PDMS	Polydimethylsiloxane	Polidimetilsiloxano
PFE	Pressurized fluids extraction	Extracción con fluidos presurizados
PhEtOH	Phenoxyethanol	Fenoxietanol
PHN	Phenanthrene	Fenantreno
PLE	Pressurized liquid extraction	Extracción con líquidos presurizados
PM	Molecular weight	Peso molecular
PMDA	Pharmaceuticals and Medical Devices Agency	
PrP	Propylparaben	Propilparabeno
PVC	Polyvinyl chloride	Cloruro de polivinilo
PYR	Pyrene	Pireno
Q		
Q	Quadrupole	Cuadrupolo
R		
R	Correlation coefficient	Coeficiente de correlación
R^2	Determination coefficient	Coeficiente de determinación
RSD	Relative standard deviation	Desviación estándar relativa
S		
S/N	Signal-to-noise ratio	Relación señal/ruido
SAs	Suspected allergenics	Sustancias potencialmente alergénicas
SCCS	Scientific Commitee on Consumer Safety	Comité Científico para la Seguridad de los Consumidores
SIM	Selected ion monitoring	
SPME	Solid-phase microextraction	Microextracción en fase sólida
SRM	Selected ion monitoring	
T		
TCS	Triclosan	Triclosán

TQ	Triple quadrupole	Triple cuadrupolo
U		
UAE	Ultrasound Assisted Extraction	Extracción asistida por ultrasonidos
USAEME	Ultrasound-assisted emulsification-microextraction	Microextracción-emulsificación asistida por ultrasonidos
UE	European Union	Unión Europea
US	Ultrasounds	Ultrasonidos
U.S.	United States	Estados Unidos
V		
Vac	Vacuum	Vacío
Vac-SPME	Vacuum-solid-phase microextraction	Microextracción en fase sólida con vacío

I. JUSTIFICACIÓN Y OBJETIVOS

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I. JUSTIFICACIÓN Y OBJETIVOS

El principal objetivo de esta Tesis Doctoral es el desarrollo de nueva metodología analítica basada en técnicas de preparación de muestra rápidas y sencillas, que junto con el análisis cromatográfico permitan determinar de manera fiable gran cantidad de sustancias en matrices muy variadas. Se divide en tres grandes Capítulos, en función de las familias de compuestos que se estudian.

En los últimos años se aprecia una creciente preocupación social por la seguridad de los productos cosméticos, ya que cada vez son más los estudios que sugieren que algunas de las sustancias empleadas en sus formulaciones pueden tener efectos perjudiciales sobre la salud de los consumidores. Hay que destacar que estos productos son empleados a diario por toda la población, incluyendo los recién nacidos y bebés, que debido a la inmadurez de sus sistemas fisiológicos son más sensibles a las sustancias tóxicas.

La Unión Europea prohíbe o restringe el uso de muchos compuestos empleados en estos productos mediante el Reglamento EC No 1223/2009. Esta regulación entró completamente en vigor hace apenas dos años e hizo necesario que muchos fabricantes reformulasen sus productos para adaptarlos a la misma y, por supuesto, se encuentra en continua revisión. Por esto y porque la mayoría de los métodos analíticos han quedado obsoletos, se hace necesario el ***desarrollo de metodología analítica*** que sea capaz de ***determinar con rapidez y fiabilidad*** un ***gran número de sustancias*** en un amplio rango de ***productos cosméticos*** y este ha sido el principal objetivo del ***Capítulo I*** de esta Tesis Doctoral. En este sentido se han desarrollado varios métodos analíticos para determinar sustancias de naturaleza química tan variada como fragancias, conservantes, plastificantes… en un amplio rango de matrices cosméticas, incluyendo productos destinados exclusivamente a la población infantil. Todos los métodos propuestos emplean técnicas de extracción rápidas, sencillas y de bajo coste que apenas consumen disolventes orgánicos como son la *dispersión de matriz en fase sólida, MSPD* y la *extracción con líquidos presurizados, PLE*. Para la determinación se ha empleado la cromatografía de masas-espectrometría de masas (GC-MS, GC-MS/MS).

El ***Capítulo II*** se centra en el desarrollo de nueva metodología analítica para el estudio de fungicidas en vino y en bagazo. El planteamiento de este trabajo se basa en que el cultivo de la vid en Galicia es un pilar fundamental de su agricultura, y los vinos obtenidos tienen un reconocido prestigio tanto nacional como internacional. Pero en muchas zonas la climatología favorece la proliferación de enfermedades del viñedo, que reducen su producción, ocasionando grandes pérdidas económicas para el sector. En estos casos, la aplicación de plaguicidas, especialmente de fungicidas, permite minimizar o evitar estos posibles daños. El problema surge cuando las sustancias químicas que se aplican sobre la vid llegan a los productos de consumo como el vino o a los sub-productos que se generan durante su elaboración, como el caso del bagazo que, además, puede servir de base para aplicaciones cosméticas o farmacéuticas. Por ello, ***se han desarrollado dos métodos de análisis en vino y en bagazo de fungicidas muy empleados en Galicia***. Las técnicas de extracción, *extracción-emulsificación asistida por ultrasonidos, USAEME*, y PLE se seleccionaron en base a la naturaleza líquida o sólida de la muestra, respectivamente. Ambas consumen cantidades mínimas de disolvente y apenas generan residuos. La determinación también se ha llevado a cabo mediante GC-MS.

En el ***Capítulo III*** se plantea el ***estudio de*** los ***hidrocarburos aromáticos policíclicos*** (PAHs), considerados como contaminantes prioritarios, ***en superficies de caucho reciclado*** utilizadas en la fabricación de parques infantiles tanto exteriores como interiores, campos de fútbol o guarderías para evitar que los niños se hagan daño. Sin embargo, cada vez son más los estudios que demuestran la presencia de metales y muchas sustancias tóxicas en estas superficies. El objetivo que se planteó en este caso, fue el de analizar no solo la propia superficie de caucho, sino también el aire y agua que permanece en contacto con la misma, ya que determinadas condiciones pueden favorecer una transferencia de las sustancias tóxicas desde las superficies. Siguiendo con el objetivo de emplear técnicas de extracción que cumplan en la medida de lo posible los principios de la "Química Verde" se ha utilizado la *microextracción en fase sólida, SPME* que no consume disolventes orgánicos.

En este Capítulo, además, se exponen los resultados obtenidos durante la estancia de investigación realizada en la *Technical University of Crete*. Tanto para los griegos como para los españoles, el aceite de oliva tiene una gran importancia ya que se considera una de las bases de la denominada "dieta mediterránea" sin embargo, numerosos estudios han demostrado la presencia de PAHs. El objetivo que se planteó fue el de ***desarrollar una nueva técnica de extracción basada en la SPME bajo condiciones de vacío para la determinación de PAHs en aceites de oliva***. Esto implica estudiar como afectan distintos parámetros al procedimiento experimental y para ello, es necesario seleccionar las condiciones que resulten más favorables para obtener una extracción eficaz.

II. INTRODUCCIÓN

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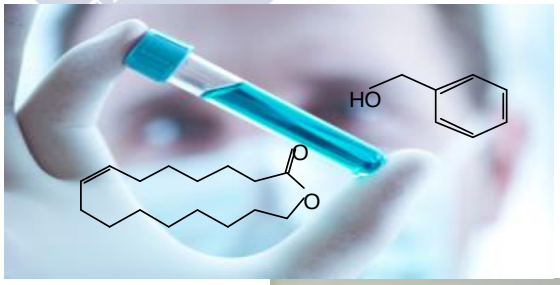
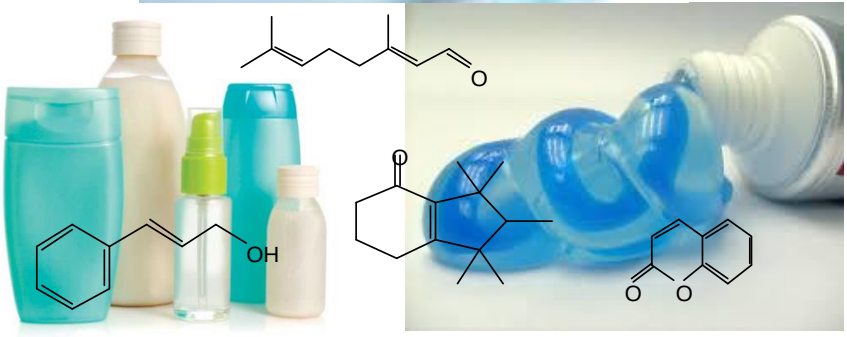
CAPÍTULO I. Sustancias potencialmente tóxicas en productos de cuidado personal





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II. INTRODUCCIÓN

CAPÍTULO I. SUSTANCIAS POTENCIALMENTE TÓXICAS EN PRODUCTOS DE CUIDADO PERSONAL

1. OBJETIVOS

La legislación que se ocupa del control de la seguridad de los productos cosméticos en Europa, el Reglamento EC No 1223/2009[1], se encuentra en continua revisión restringiendo y/o prohibiendo el uso de determinadas sustancias en la formulación de los mismos. Por eso es necesario disponer de métodos analíticos fiables y robustos que permitan la detección de estos compuestos, ya que los métodos oficiales son escasos y han quedado en muchos casos obsoletos.

El ***objetivo*** de este Capítulo es el de ***desarrollar métodos de análisis*** para ***determinar*** una ***gran variedad de sustancias químicas*** de diversa naturaleza ***en un amplio rango de matrices cosméticas***. Para ello se emplearán técnicas de extracción sencillas y rápidas como la dispersión de matriz en fase sólida (MSPD) y la extracción con líquidos presurizados (PLE), según la naturaleza de las muestras. Para la determinación de los analitos se empleará la cromatografía de gases acoplada a la espectrometría de masas como técnica de detección (GC-MS). También se empleará la espectrometría de masas en tándem (MS/MS), ya que permite mejorar la sensibilidad y la selectividad, minimizando las posibles interferencias que puedan causar las matrices cosméticas ya que estas pueden contener sustancias con estructuras químicas similares a los compuestos de interés que interfieran en su detección. Cabe destacar que esta es la primera vez que GC-MS/MS se ha empleado para analizar simultáneamente fragancias alergénicas y conservantes en productos de cuidado personal.

Durante la optimización de la etapa de extracción, se han aplicado diseños experimentales para obtener la máxima eficacia; asimismo, toda la metodología desarrollada ha sido validada mediante herramientas estadísticas, demostrándose la fiabilidad de la misma en términos de exactitud y precisión, y tanto los límites de detección como los de cuantificación (LODs y LOQs) estuvieron en todos los casos muy por debajo de los requerimientos legales.

2. PRODUCTOS DE CUIDADO PERSONAL Y COSMÉTICOS. DEFINICIÓN

Se entiende por producto de cuidado personal (***personal care product, PCP***) todas las sustancias químicas incluidas en diferentes productos de uso diario como pastas de dientes, perfumes, lociones corporales, champús... Sin embargo, este término no está definido por ley y ya que la mayoría de los productos denominados así son cosméticos, ambos términos serán empleados como sinónimos.

La actual regulación vigente en Europa para este tipo de productos, EC No 1223/2009[1] , en su artículo 2 apartado a) define como ***producto cosmético*** "toda sustancia o mezcla destinada a ser puesta en contacto con las partes superficiales del cuerpo humano (epidermis, sistema piloso y capilar, uñas, labios y órganos genitales externos) o con los dientes y las mucosas bucales, con el fin exclusivo o principal de limpiarlos, perfumarlos, modificar su aspecto, protegerlos, mantenerlos en buen estado o corregir los olores corporales".

Griegos y romanos ya empleaban perfumes con bases aceitosas y pigmentos sobre la piel con fines decorativos. Hace 5000 años, los egipcios aplicaban pigmentos verdes y negros sobre los párpados para protegerlos del sol. En el siglo XVII, los cortesanos de Luis XIV en Francia, coloreaban sus rostros con sustancias naturales como azafrán o pólen, a la vez que en Europa se empezaban a emplear perfumes, los cuales tuvieron una gran popularidad en el siglo XVIII[2]. Desde principios del siglo XX hasta la actualidad la ***industria cosmética*** ha experimentado un importante crecimiento, debido a una demanda cada vez mayor de productos no solo con fines decorativos, sino también para su uso como productos de higiene. Por todo ello, los productos cosméticos son empleados hoy en día por millones de personas en todo el mundo y son una ***importante balanza comercial*** en los países más industrializados. En el año 2013, la facturación de productos cosméticos en Europa ascendió a 69 billones de euros, seguida por Estados Unidos, China y Japón con 47, 29 y 18 billones, respectivamente. Es importante destacar que ***España*** se sitúa como ***quinta potencia europea*** con un volumen de producción de 6,4 billones de euros. En cuanto a las exportaciones, el mercado europeo representa una tercera parte del global y en este campo, el mercado español también se sitúa en quinta posición, con exportaciones por valor de 2,5 billones de euros. Actualmente, en el mercado común europeo se comercializan más de 350.000 cosméticos, formados por unas 13.000 sustancias[3,4].

La mayoría de los productos cosméticos tienen una vida útil menor de cinco años y los fabricantes reformulan el 25% de sus productos cada año, para mantenerse a la vanguardia en un mercado altamente competitivo. Está claro que la ***innovación*** impulsa la industria cosmética para ofrecer ***productos originales*** que los hagan más atractivos a los consumidores pero sin dejar de lado la seguridad. La legislación que concierne a estos productos está en continua revisión, restringiendo y/o prohibiendo el uso de determinadas sustancias en su formulación.

En los últimos años, muchos fabricantes están uniendo ciencia y naturaleza mediante el uso de ingredientes "naturales" y cada vez más marcas ofrecen productos libres de ciertas sustancias (fragancias, colorantes, etc). Aunque se han creado certificados tanto a nivel internacional (NATRUE, IMO CONTROL) como nacional: COSMEBIO y ECOCERT (Francia), ORGANIC SOIL ASSOCIATION (Reino Unido), o BHID (Alemania) para distinguir a estos productos naturales de los convencionales, actualmente no existe en ningún país del mundo una definición oficial de ***cosmética natural*** y, la base de muchos de los cosméticos que emplean los términos *natural*, *ecológico* u *orgánico*, está formada por compuestos químicos, aunque en la composición final del producto predominen las sustancias de origen vegetal. A pesar de esto, de cara al consumidor, la presencia de ingredientes "naturales" se considera hoy en día una cualidad positiva[5,6].

3. CLASIFICACIÓN

Dentro de los productos cosméticos se incluyen: cremas, emulsiones, lociones, geles y aceites para la piel, jabones, perfumes, geles de baño y ducha, desodorantes y antitranspirantes, colorantes para el cabello, productos para la limpieza del cabello así como para su mantenimiento, maquillaje y productos para desmaquillar, productos destinados a aplicarse en los labios, para el

cuidado dental y bucal, para el cuidado de las uñas, productos de higiene íntima, protectores solares, anti-arrugas y muchos otros[1].

Los productos cosméticos se pueden clasificar:

a) En función de su forma cosmética: cremas, suspensiones, soluciones, polvos, aerosoles, espumas, vaporizadores, geles, barras, lápices, pastillas, sales, perlas, máscaras, envases monodosis, soportes impregnados, roll-on…

b) En función de su lugar de aplicación: sobre la piel, ojos, labios, anexos epidérmicos…

c) En función de su uso principal: higiene, mantenimiento, protección de la piel, tratamiento de las alteraciones de la misma…

Asimismo, la legislación diferencia entre productos de permanencia o ***leave-on*** ("producto cosmético destinado a permanecer en contacto prolongado con la piel, el pelo o las mucosas") y productos de aclarado o ***rinse-off*** ("producto cosmético destinado a ser eliminado tras su aplicación en la piel, el pelo o las mucosas").

4. LEGISLACIÓN

La legislación varía mucho de un país a otro, pero hoy en día la mayoría de ellos tienen algún tipo de normativa formal que, o bien restringe o bien prohíbe determinados ingredientes presentes en las formulaciones. En ***España***, la regulación sobre productos cosméticos ha estado recogida en el Real Decreto del 17 de octubre de 1977 (***Directiva 76/768/CEE***) que recopiló en un solo texto toda la normativa existente hasta el momento y la adaptó a la legislación comunitaria de entonces. En ese Decreto, se incluía la definición de producto cosmético, así como las condiciones técnico-sanitarias que debían reunir, su control sanitario, los requisitos de las instalaciones donde se elaboraban y las de importación de productos de terceros países, así como etiquetado, publicidad, sanciones e infracciones.

La aparición en 2008 de nueva normativa comunitaria sobre clasificación, etiquetado y envasado de productos cosméticos hizo necesario modificar el Real Decreto de 1977 para adaptarlo a esta nueva normativa e incorporar al ordenamiento español los criterios de clasificación y etiquetado de sustancias y mezclas del Sistema Mundialmente Armonizado de Clasificación y Etiquetado de Productos Químicos (cuyas siglas en inglés se corresponden con GHS), adoptado a escala internacional en el marco de las Naciones Unidas, así como para armonizar íntegramente las normas comunitarias a fin de lograr un mercado interior para los productos cosméticos, garantizando al mismo tiempo la protección de la salud humana.

El 22 de diciembre de 2009 se publicó en el Diario Oficial de la Unión Europea el ***Reglamento (EC) No 1223/2009 del Parlamento Europeo y del Consejo de 30 de noviembre de 2009 sobre productos cosméticos***[1], que entró en vigor 20 días después, el 11 de enero de 2010, aunque la mayoría de las disposiciones no entrarían en vigencia hasta el 11 de julio de 2013, fecha en la que la Directiva 76/768/CEE fue derogada. Dicho Reglamento EC No 1223/2009 establece las "normas que

deben cumplir todos los productos cosméticos comercializados, con objeto de velar por el funcionamiento del mercado interior y lograr un elevado nivel de protección de la salud humana". Es importante destacar que este Reglamento se mantiene en constante revisión y actualización.

En ***Estados Unidos*** la regulación de estos productos se lleva a cabo mediante la ***Ley Federal de Alimentos, Medicamentos y Cosméticos de Estados Unidos*** (FD&C), conjunto de leyes aprobadas por el Congreso en 1938, y que dieron autoridad a la *Food and Drug Administration* (FDA) para supervisar la seguridad de estos productos[7]. Cabe destacar que la creación de esta ley fue inducida después de la muerte de más de 100 personas, tras haber empleado un elixir con sabor a frambuesa, el cual había sido introducido en el mercado sin las pruebas correspondientes (posteriormente se demostró que alrededor del 70% del elixir era dietilenglicol, un compuesto altamente tóxico). Para los productos importados, estos deben seguir las mismas leyes y regulaciones que en el caso de que fuesen producidos en ese país.

Llama poderosamente la atención que la legislación europea es mucho más restrictiva en cuanto a ingredientes permitidos en la formulación de productos cosméticos que la americana. Frente a las diez sustancias prohibidas por la FDA americana, en Europa a fecha de hoy hay en torno a 1370 cuyo uso en cosméticos no está permitido y 280 que se encuentran restringidas, aunque hay que destacar que productos que en Europa se consideran cosméticos, en Estados Unidos pueden estar clasificados como drogas, según el uso al que estén destinados (champús anticaspa, pastas de dientes con flúor o desodorantes-antitranspirantes son considerados tanto cosméticos como drogas). Asimismo, en Europa está prohibido desde 2009 comercializar productos cosméticos cuya formulación final, ingredientes o combinaciones de ingredientes hayan sido experimentados en animales, mientras que en Estados Unidos se sigue permitiendo su uso.

En ***Canadá*** existe una *Cosmetic Ingredient Hotlist* que es continuamente revisada y actualizada, donde se recogen los ingredientes prohibidos o restringidos para su empleo en productos cosméticos[8]; fue creada con la intención de constituir una herramienta útil para los fabricantes a la hora de comercializar sus productos; además, en la sección 16 de la ***Food and Drug Act***, se encuentran entre otras, las disposiciones relativas al etiquetado y almacenamiento de estos productos[9]. En ***Japón***, la regulación de estos productos se lleva a cabo mediante la PMDA (***Pharmaceuticals and Medical Devices Agency***) a través de la Ley sobre Productos Farmacéuticos[10].

Teniendo en cuenta estas legislaciones, se creó en 2007 el ICCR (*International Cooperation on Cosmetics Regulation*), un grupo de cooperación internacional de autoridades reguladoras de productos cosméticos compuesto por Estados Unidos (*FDA-Food and Drug Administration*), la Unión Europea (*European Commision, DG Enterprise*), Japón (*MHLW-Ministry of Health, Labour, and Welfare*) y Canadá (*Health Canada*). Esta estructura reguladora multilateral vela por los altos niveles de protección al consumidor a la vez que trata de minimizar las barreras para el comercio internacional.

5. GRUPOS DE SUSTANCIAS ESTUDIADAS

A continuación se presentan los compuestos estudiados en este trabajo. Se dividen en tres grandes familias que engloban ***cerca de 70 sustancias*** químicas que se pueden encontrar en las formulaciones de productos cosméticos y de cuidado personal; la mayoría están ***reguladas*** por la normativa europea[1] debido a sus ***potenciales efectos nocivos*** sobre la salud.

5.1. FRAGANCIAS

Uno de los atributos que más valoran los consumidores al escoger un producto cosmético o de cuidado personal (especialmente en el caso de los perfumes) es su olor, su fragancia. Realmente las fragancias son el resultado de una mezcla de sustancias odoríferas (existen más de 2500 ingredientes que se emplean para elaborar fragancias) con identidad única que dan una percepción sensorial identificable[11]. Se encuentran presentes además, en otros productos de uso mayoritario como alimentos, bebidas, ambientadores… y su principal función es la de proporcionar olores agradables y atrayentes para el consumidor.

En este estudio se incluyen dos grandes grupos de fragancias, las fragancias alergénicas (*supected allergenics*, SAs) y los almizcles sintéticos (*musks*). A continuación se presenta una breve descripción de ambas familias.

5.1.1. FRAGANCIAS ALERGÉNICAS

5.1.1.1. Introducción y clasificación

Un cálculo estimado dice que entre el 1-3% de la población sufre alergias y dermatitis debido a las fragancias presentes en los productos cosméticos; de hecho 26 de ellas fueron calificadas por la Unión Europea como ***"fragancias alergénicas"***.

De estos 26 compuestos, 2 son extractos naturales, obtenidos a partir de líquenes: los conocidos como ***oak moss*** (*Evernia Prunastri*) y ***tree moss*** (*Evernia Furfuracea*), cuyos compuestos químicos principales son atranol y cloroatranol (actualmente el Comité Científico Europeo para la Seguridad de los Consumidores (*Scientific Committee on Consumer Safety, SCCS*) propone prohibir su uso en la formulación de productos cosméticos); las otras 24 se definen químicamente como volátiles y están formadas por compuestos de naturaleza química tan diversa como terpenos, terpenoides, ésteres, alcoholes, aldehídos….

Esta Tesis Doctoral, se ha centrado en el estudio de esas 24 fragancias alergénicas volátiles, cuyo número identificativo CAS, peso molecular (Pm) y algunas características físicas, así como su estructura se presentan en la Tabla I.1.

Además, se han incluido otras dos fragancias: ***pinene*** que, aunque inicialmente no estuvo considerada como fragancia alergénica, desde 2013 se considera como tal y ***methyleugenol***, que durante años estuvo incluida entre las sustancias completamente prohibidas en productos cosméticos (Anexo II de la Regulación (EC) No 1223/2009), pero en una posterior revisión de este Reglamento, fue incluida en el Anexo III (sustancias permitidas con restricciones). Sus características también se muestran en la Tabla I.1.

Tabla I.1. Nombres, CAS, propiedades físicas y estructura de las fragancias alergénicas estudiadas.

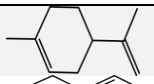
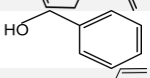
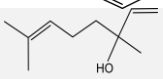
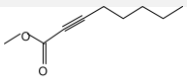
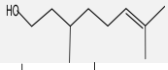
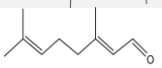
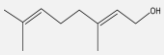
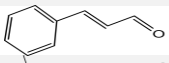
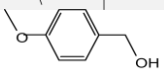
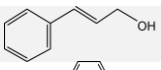
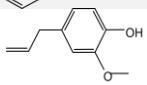
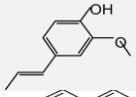
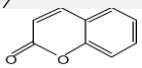
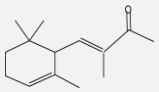
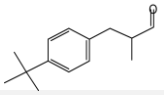
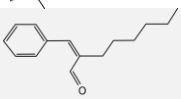
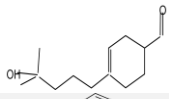
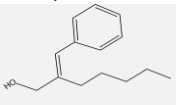
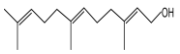
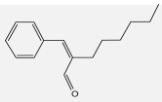
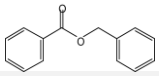
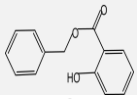
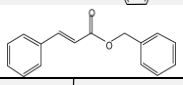
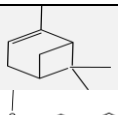
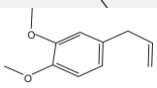
Nombre común	Nombre químico	Número CAS	Pm	log K_{ow}	Punto de ebullición (ºC)	Estructura
Limonene	(4R)-1-Metil-4-(1-metiletenil)ciclohexeno	5989-27-5	136	4,57	176	
Benzyl Alcohol	Hidroximetilbenceno	100-51-6	108	1,05	205	
Linalool	3,7-Dimetil-1,6-octadien-3-ol	78-70-6	154	3,28	198	
Methyl-2-octynoate	Metilheptin carbonato	111-12-6	154	2,60	219	
Citronellol	(±)-3,7-Dimetioct-6-en-1-ol	106-22-9	156	3,38	225	
Citral	3,7-Dimetil-2,6-octadienal	5392-40-5	152	3,17	229	
Geraniol	3,7-Dimetil-(2E)-2,6-octadien-1-ol	106-24-1	154	3,28	229	
Cinnamal	3-Fenil-2-propenal	104-55-2	132	2,22	252	
Hydroxycitronellal	7-Hidroxi-3,7-dimetiloctanal	107-75-5	172	1,54	240	
Anise Alcohol	4-Metoxibencil alcohol	105-13-5	138	1,10	259	
Cinnamyl alcohol	3-Fenil-2-propen-1-ol	104-54-1	134	1,93	250	
Eugenol	2-Metoxi-4-(2-propenil)-fenol	97-53-0	164	2,20	256	
Isoeugenol	2-Metoxi-4-(1-propenil)-fenol	97-54-1	164	2,45	267	
Coumarin	2H-1-benzopiran-2-one	91-64-5	146	1,39	298	
α-isomethylionone	3-Metil-4-(2,6,6-trimetil-2-ciclohexen-1il)-3buten -2- ona	127-51-5	206	4,41	266	
Lilial®	2-(4-*terc*-butilbencil) propionaldehido	80-54-6	204	4,07	279	
Amylcinnamal	2-Bencilideneheptanal	122-40-7	202	4,80	289	
Lyral®	Hidroxihexil-3ciclohexeni-carboxaldehido	31906-04-4	210	2,53	319	
Amylcinnamyl alcohol	2-Pentil-3-fenilprop-2-en-1-ol	101-85-9	204	4,37	250	

Tabla I.1. Continuación

Nombre común	Nombre químico	Número CAS	Pm	log K_{ow}	Punto de ebullición (ºC)	Estructura
Farnesol	3,7,11-trimetildodeca-2,6,10-trien-1-ol	4602-84-0	222	5,31	283	
Hexylcinnamal	2-Bencilidenoctanal	101-86-0	216	4,82	308	
Benzyl benzoate	Fenilmetil benzoato	105-13-5	212	3,97	324	
Benzyl salicylate	Bencil-2-hidroxibenzoato	118-58-1	228	4,31	320	
Benzyl cinnamate	3-Fenil-2-propenoic-fenilmetil ester	103-41-3	238	3,65	371	
Pinene	Biciclo(3.1.1)hept- 2-ene, 2,6,6-trimetil	80-56-8	136	4,37	155	
Methyleugenol	1,2-Dimetoxi-4-(2-propenil)-benceno	93-15-2	178	2,9	248	

Es importante destacar que desde que en 1999 se identificaron estas primeras 26 fragancias alergénicas, la lista ha ido aumentando; ***actualmente***, además de las 26 ya nombradas, el SCCS identifica unas ***56 sustancias*** (30 compuestos químicos y 26 extractos naturales) reconocidas como "alérgenos de contacto establecidos en seres humanos"[12].

5.1.1.2. Usos y aplicaciones

Según la industria cosmética, el 80% de la producción total de fragancias alergénicas se destina a la fabricación de cosméticos y el 90% de las que se producen en volúmenes > 175 toneladas/año son empleadas en la ***formulación de perfumes***[12]. En estos casos, la concentración de fragancias alergénicas puede llegar hasta un 30%, mientras que en otros productos como desodorantes, el porcentaje de fragancia varía entre el 0,1-1%.

Diferentes estudios realizados en los últimos años han revelado que la principal vía de contacto con las fragancias alergénicas es a través de los productos cosméticos y de cuidado personal, pero hay que tener en cuenta que estas sustancias también se encuentran presentes en otros productos de consumo masivo como alimentos, bebidas, detergentes, suavizantes, productos de limpieza, e incluso en algunos medicamentos de uso tópico, por lo que la exposición a las 26 fragancias alergénicas se hace más que evidente en la vida diaria. Estos estudios revelan también que la mayoría de los cosméticos contienen combinaciones de 3-4 alérgenos por producto. Asimismo, muchos de los productos etiquetados como "libres de fragancias" pueden contenerlas, ya sea por el uso de aromas o por el uso de sustancias naturales[13-17].

Estos compuestos también se emplean en productos destinados a la población infantil, como es el caso de los juguetes. Debido a la creciente preocupación en cuanto a sus efectos sobre la salud de los más pequeños, recientemente la Unión Europea prohibió el uso de 55 fragancias

alergénicas en la fabricación de los mismos, y exigió la inclusión de otras 11 en las etiquetas, cuando superen el 0,01% en peso del juguete[18,19].

5.1.1.3. Distribución en el medio ambiente

Debido a sus rangos de presiones de vapor (10-10^{-5} Pa) y a su amplio rango de solubilidad en agua (10^{3}-10^{-1} mg·L^{-1}) estos compuestos se distribuyen rápidamente por el medio ambiente, a través de las ***aguas residuales*** tras la eliminación de los productos cosméticos y de cuidado personal por el desagüe. Por lo tanto, el destino final de estas sustancias, además del agua puede ser cualquiera de los ***receptores medioambientales*** (aire, sedimentos, biota…), lo que supone una concentración final considerable en el medio, incluso si los compuestos son biodegradables. Por ejemplo, para el caso del limoneno, que no presenta grupos funcionales para su hidrólisis y su anillo ciclohexeno y grupo etileno son resistentes a la misma, tiene una vida media de más de 1000 días en el medio acuático; asimismo, su factor de bioconcentración es de 246-262, lo que implica que es un compuesto fácilmente acumulable en peces u otros organismos acuáticos[20-22].

5.1.1.4. Efectos sobre la salud

Como ya se ha comentado, un cálculo estimado dice que entre el 1-3% de la población sufre ***alergia*** a los productos cosméticos debido a estas sustancias. La alergia se produce cuando un individuo se expone (ya sea por contacto directo o por inhalación) a productos que contienen este tipo de fragancias. Las zonas comúnmente más afectadas son: cara, manos y axilas, ya que son las más expuestas a estos productos. La alergia de contacto es una patología que altera el sistema inmunológico; esto quiere decir que una vez que la alergia se desarrolla, las células del sistema inmunitario reconocen y reaccionan frente al alérgeno. Como consecuencia de esto, los síntomas de una ***dermatitis de contacto*** (eczemas, sequedad de la piel, picor...) suelen darse después de una exposición continuada a la sustancia; se calcula que el 10% de la población europea con eczemas presenta algún tipo de alergia de contacto a los cosméticos. Además, estos compuestos pueden empeorar otras enfermedades cuyo origen no sea el contacto directo con las mismas (rinitis, asma, sinusitis…) y este efecto sinérgico se puede producir cuando la exposición sea a valores de concentración inferiores a los necesarios para provocar la misma reacción en una persona sana[23,24].

5.1.1.5. Regulación en productos cosméticos

Debido a sus posibles efectos negativos para la salud, la legislación europea exige la ***presencia*** de estos compuestos en la ***lista de ingredientes*** cuando sus niveles de ***concentración*** en el producto terminado se encuentren por ***encima del 0,01% (100 µg·g^{-1}) o 0,001% (10 µg·g^{-1})*** según se trate de un producto de ***aclarado*** (gel de ducha, champú) o de ***permanencia en la piel*** (loción hidratante, maquillaje), respectivamente[1]. Asimismo, tres de ellas (*hydroxycitronellal*, *methyleugenol* e *isoeugenol*) presentan restricciones en cuanto a sus máximas concentraciones permitidas en el producto final y otra, *lyral®*, se encuentra actualmente propuesta por el SCCS para su inclusión en el Anexo II de la Regulación (sustancias prohibidas) ya que considera que una exposición continuada a este compuesto no es segura para los consumidores, incluso a bajas concentraciones[25]. En la Tabla I.2 se muestran las prohibiciones y restricciones en términos de máxima concentración permitida para estas fragancias.

Tabla I.2. Fragancias prohibidas o restringidas en términos de máxima concentración permitida

Nombre común	Prohibiciones y restricciones[1]
Lyral®	Propuesta para ser prohibida
Hydroxycitronellal	1%*
Methyleugenol	0,01% (fragancia fina); 0,004% (agua de colonia); 0,002% (crema de fragancia); 0,0002% (otros productos de permanencia); 0,001% (productos de aclarado).*
Isoeugenol	0,02%*

* % referido al producto terminado

5.1.2. ALMIZCLES SINTÉTICOS (MUSKS)

5.1.2.1. Introducción y clasificación

Almizcle fue el nombre dado originalmente al perfume obtenido a partir de una sustancia de fuerte olor segregada por una glándula de ciertas plantas y/o animales, entre los que se encuentra el ciervo almizclero (*Moscus Moschiferu)*. Los principales componentes químicos de los almizcles naturales son una cetona macrocíclica denominada Muscona (de ahí el nombre en inglés, ***musk***), cuya estructura se muestra en la Figura I.1 y una piridina macrocíclica, la Muscopiridina.

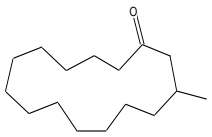


Figura I.1. Estructura química de Muscona

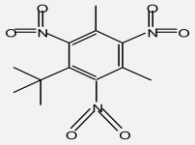
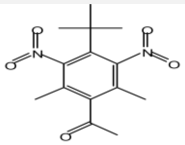
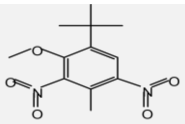
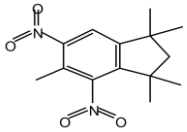
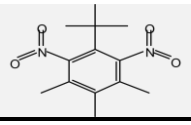
Ya que la mayoría de los animales y plantas de los que se extrae la glándula se encuentran en peligro de extinción y, debido a su alto coste de producción, hoy en día los almizcles naturales casi no se emplean y se han sustituido por aromas químicos sintéticos que imitan el olor de los naturales, ofreciendo un aroma característico que determina el olor del producto final, además de una notable persistencia tanto en piel como en otros tejidos[26-28].

Según su estructura química, los almizcles o *musks* se pueden dividir en tres grandes grupos: nitrogenados, policíclicos y macrocíclicos.

- Almizcles nitrogenados

También conocidos como "*nitromusks*" o "nitroalmizcles", este grupo está formado por cinco compuestos ***derivados del di-, tri-nitrobenceno***. En la Tabla I.3 se presentan sus nombres, números CAS, algunas propiedades físicas y sus estructuras.

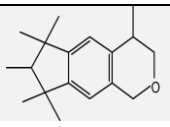
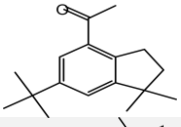
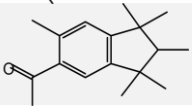
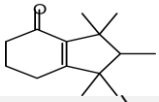
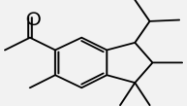
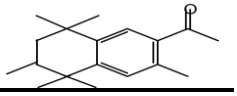
Tabla I.3. Nombres, CAS, propiedades físicas y estructuras de las *nitromusks* estudiadas.

Nombre común	Nombre químico	CAS	Pm	log K_{ow}	Estructura
Musk Xylene	1-terc-butil-3,5-dimetil-2,4,6-trinitrobenceno	81-15-2	297	4,9	
Musk Ketone	4-terc-butil-3,5-dinitro-2,6-dimetilacetilbenceno	81-14-1	294	4,3	
Musk Ambrette	6-terc-butil-3-metil-2,4-dinitroanisol	83-66-9	268	4,2	
Musk Moskene	1,1,3,3,5-pentametil-4,6-dinitro-2H-indeno	116-66-5	278	5,3	
Musk Tibetene	1-terc-butil-3,4,5-trimetil-2,6-dinitrobenceno	145-39-1	266	5,0	

- Almizcles policíclicos

También denominadas "*polimusks*", pertenecen a este grupo las sustancias cuya ***estructura química básica*** son las moléculas de ***indano y tetralina*** con un gran número de sustituyentes, principalmente grupos metilo. En la Tabla I.4 se muestran las estudiadas.

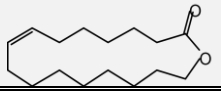
Tabla I.4. Nombres, CAS, propiedades físicas y estructuras de las *polimusks* estudiadas.

Nombre común	Nombre químico	CAS	Pm	log K_{ow}	Estructura
Galaxolide	1,3,4,6,7,8-hexahidro-4,6,6,7,8,8-hexametil ciclopenta(g)-2-benzopireno	1222-05-5	258	5,3	
Celestolide	4-acetil-6-terc-butil-1,1-dimetilindano	13171-00-1	244	5,4	
Phantolide	6-acetil-1,1,2,3,3,5-hexametilindano	15323-35-0	244	6,7	
Cashmeran	1,1,2,3,3-pentametil-2,5,6,7-tetrahidroinden-4-ona	33704-61-9	206	4,9	
Traseolide	5-acetil-3-isopropil-1,1,2,6-tetrametilindano	68140-48-7	258	8,1	
Tonalide	6-acetil-1,1,2,4,4,7-hexametiltetralina	1506-02-1	258	5,7	

- Almizcles macrocíclicos

El principal inconveniente que presentan los almizcles nitrogenados y policíclicos es su lenta degradación, por lo que se encuentran muy diseminados en el medio; por esto y por sus efectos nocivos sobre la salud, su uso ha descendido en los últimos años y se han sustituido por un tercer grupo, los almizcles macrocíclicos, compuestos por mezclas de sustancias sintéticas y naturales. Básicamente son cetonas macrocíclicas (origen animal), lactonas y bis-lactonas (origen vegetal). Aunque su producción es fácil, tienen un elevado coste de producción pero presentan ***mayor biodegradabilidad*** que las "*nitromusks*" y "*polimusks*", por lo que son las menos perjudiciales para la salud y el medio ambiente[28]. Dentro de esta categoría se encuentra la Ambrettolide, cuyas propiedades físicas y estructura se muestra en la Tabla I.5.

Tabla I.5. Nombre, CAS, propiedades físicas y estructura del almizcle macrocíclico estudiado.

Nombre común	Nombre químico	Número CAS	Pm	log K_{ow}	Estructura
Ambrettolide	17-oxacicloheptadec -6-en-1-ona	7779-50-2	252	5,4	

5.1.2.2. Usos y aplicaciones

La principal aplicación de los almizcles se centra sin duda en la industria cosmética, destacando la elaboración de ***perfumes***. Estas sustancias se encuentran también en otros productos de consumo como detergentes, suavizantes, productos de limpieza del hogar, ambientadores, herbicidas, aditivos del tabaco…

- Almizcles nitrogenados

Musk Xylene fue la primera en ser sintetizada por Albert Baur en 1888; más tarde se sintetizaron las demás. Las *nitromusks* fueron las primeras en ser introducidas en el mercado a finales del siglo XX, pero debido a sus problemas toxicológicos su producción ha ido decreciendo exponencialmente durante las últimas décadas. Solamente dos de ellas siguen teniendo importancia, Musk Ketone y Musk Xylene; estas, junto con otras dos *polimusks* (Galaxolide y Tonalide) representaban en 2005 el 95% del mercado europeo de almizcles sintéticos[29].

Mientras que Musk Ketone tiene su principal aplicación en la ***industria cosmética***, Musk Xylene se emplea mayoritariamente en la ***formulación de detergentes*** y productos de limpieza. Cabe destacar que en estos últimos casos, la adición de las fragancias no contribuyen en nada a la detergencia de la formulación; simplemente ofrecen un atractivo estético, disimulando olores desagradables de otros ingredientes en la suciedad del agua de lavado.

- Almizcles policíclicos

Este grupo de fragancias se desarrolló a partir de 1950 y poco a poco han ido reemplazando a las nitrogenadas y, aunque su síntesis industrial es más costosa, a finales del siglo XX su producción llegó a alcanzar el ***70% de la producción mundial*** del mercado de fragancias sintéticas.

- Almizcles macrocíclicos

Debido a su estructura, se puede producir una fácil descomposición microbiana de las mismas, convirtiéndolas en compuestos de estabilidad química reducida y mayor biodegradabilidad que los grupos anteriores, lo que las convierte en ***menos perjudiciales*** para el ser humano y el medio ambiente.

5.1.2.3. Distribución en el medio ambiente

Los elevados valores de log K_{OW} que se muestran en las Tablas I.3, I.4 y I.5, demuestran una alta solubilidad de estos compuestos en disolventes orgánicos y una ***alta persistencia en los tejidos grasos*** y biológicos; además son compuestos químicamente muy estables, característica que determina su alta persistencia una vez introducidas ***en el medio ambiente***[30]. Tanto es así, que se han encontrado ampliamente distribuidas tanto en sistemas acuáticos y marinos, como en la atmósfera o en el aire interior[31-33].

Además, al formar parte de muchos productos de uso doméstico que se eliminan a través de sistemas de desagüe, se han encontrado almizcles sintéticos en aguas de alcantarillado, residuales y agua de mar, destacando en algunos casos las elevadas concentraciones de Galaxolide y Tonalide. También se han encontrado en aguas de lluvia; llama la atención que Musk Ambrette (prohibida en la formulación de cosméticos desde 1995) se encontró en el 34% del agua de lluvia de los puntos de recogida, en un estudio realizado en Holanda en 2003[34].

5.1.2.4. Efectos sobre la salud

Debido a su uso masivo en productos cosméticos de uso diario y en productos de limpieza, la vía de contacto principal de estos compuestos es por absorción a través de la piel y en menor medida por inhalación y por ingestión[35,36].

Como ya se ha comentado, este tipo de fragancias sintéticas se ha desarrollado para sustituir a las naturales, por lo que tratan de imitar su olor (el cual en algunas especies ***animales*** es debido a las feromonas); por lo tanto, si se introducen en grandes cantidades en el medio ambiente, pueden producir ***aturdimiento o confusión*** entre los animales, alterando su sistema reproductor y endocrino. Además, cada vez existen más evidencias científicas de que algunas nitromusks y polimusks, incluyendo las empleadas habitualmente en perfumes pueden ser capaces (ya sea como compuestos principales o como metabolitos) de interferir con la hormona que regula los sistemas de comunicación de peces, anfibios y mamíferos[37,38].

En el caso de los humanos, las más nocivas son las fragancias nitrogenadas (especialmente Musk Ambrette, Tibetene y Moskene) que se detectaron por primera vez en tejidos adiposos en los años 90, demostrándose posteriormente sus ***efectos genotóxicos y neurotóxicos*** (estudios recientes las catalogan como sospechosas de tener efectos cancerígenos). Además, estos compuestos presentan una gran capacidad de concentración en tejidos vivos. Tanto es así que se han encontrado contaminando la sangre humana y leche materna[39-41].

5.1.2.5. Regulación en productos cosméticos

Estos compuestos no aparecen como tal en el ***etiquetado*** de cosméticos y productos de cuidado personal, sino que lo hacen bajo el término ***fragancia*** *(fragrance)* o ***perfume*** *(parfum)*. Según la actual legislación europea[1], las *musks* nitrogenadas ***Ambrette, Tibetene y Moskene*** fueron las primeras en ser ***prohibidas*** en 1995 para su uso en la formulación de productos cosméticos debido a su neurotoxicidad y genotoxicidad, mientras que Musk Ketone y Musk Xylene están permitidas pero con restricciones como se muestra en la Tabla I.6.

En cuanto a las fragancias policíclicas, y aunque no se ha demostrado su toxicidad, su comportamiento químico es muy parecido a la Versalide (fragancia prohibida a finales de los años 70 debido a su neurotoxicidad), por lo que se han establecido restricciones en el uso de algunas de ellas (Tabla I.6) mientras que la única fragancia macrocíclica incluida en este estudio no presenta actualmente ningún tipo de restricción para su empleo en la formulación de productos cosméticos.

Tabla I.6. Prohibiciones y restricciones en productos cosméticos para los almizcles estudiados.

Almizcles	Prohibiciones y restricciones[1]
	Nitrogenados
Musk Xylene	Prohibida en productos orales; 1% (fragancia fina); 0,4% (agua de colonia); 0,03% (otros productos)
Musk Ketone	Prohibida en productos orales; 1,4% (fragancia fina); 0,56% (agua de colonia); 0,042% (otros productos)
Musk Ambrette	Prohibida
Musk Moskene	Prohibida
Musk Tibetene	Prohibida
	Policíclicos
Galaxolide	n.r
Celestolide	n.r
Phantolide	2% (productos de permanencia)
Cashmeran	n.r
Traseolide	n.r
Tonalide	Prohibida en productos orales; 0,2% (productos de aclarado); 0,1% (productos de permanencia, excepto: 1% hidroalcohólicos, 2,5% fragancia fina y 0,5% crema de fragancia)
	Macrocíclico
Ambrettolide	n.r

n.r: No restringida en términos de máxima concentración permitida

5.2. PLASTIFICANTES

Dentro de este grupo, se encuentran los ftalatos y adipatos, empleados habitualmente en la formulación de productos cosméticos. A continuación se detallan sus principales características y usos, así como sus restricciones.

5.2.1. Ftalatos y adipatos

Los llamados ***ftalatos*** son ésteres del ácido ftálico (ácido 1,2-bencenodicarboxílico) con varios alcoholes. Su estructura básica se muestra en la Figura I.2.

Se sintetizan empleando anhídrido ftálico y dos moléculas del alcohol correspondiente. El ácido ftálico en general se prepara a través de una oxidación catalítica del naftaleno u orto-xileno a 400-500ºC, usando como catalizador pentóxido de vanadio. Se trata de un grupo muy amplio de compuestos, ya que existe una gran variedad de alcoholes que pueden reaccionar con el ácido ftálico para originar distintos ésteres, en los que solo varían las cadenas carbonadas (R, R')[42].

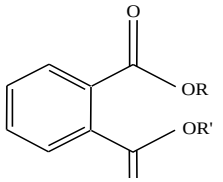


Figura I.2. Estructura básica de los ftalatos

Los ftalatos objeto de estudio, así como sus números CAS, algunas propiedades físicas y estructuras se muestran en la Tabla I.7.

Tabla I.7. Nombres, CAS, propiedades físicas y estructura de los plastificantes estudiados.

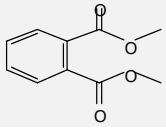
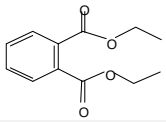
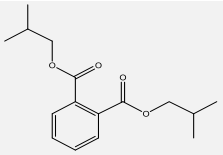
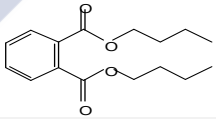
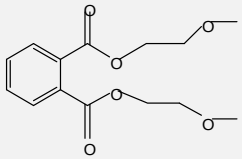
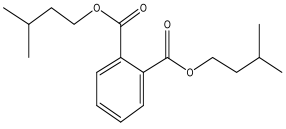
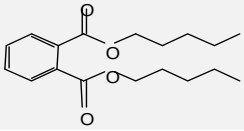
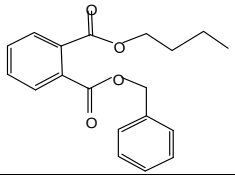
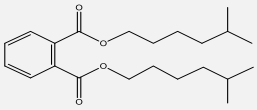
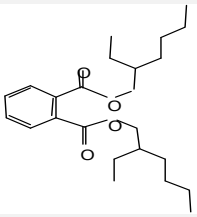
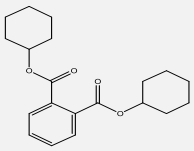
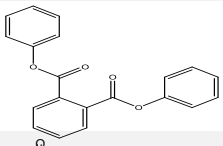
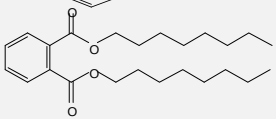
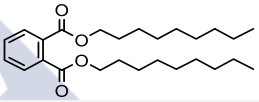
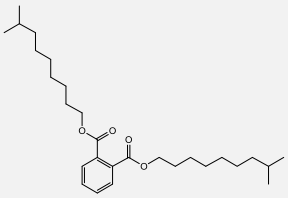
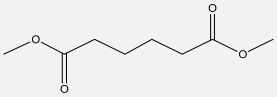
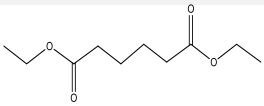
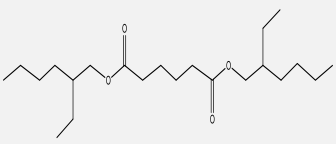
Nombre común	Abreviatura	CAS	Pm	log K_{OW}	Estructura
Ftalatos					
Dimethylphthalate	DMP	131-11-3	194	1,61	
Diethylphthalate	DEP	84-66-2	222	2,54	
Diisobutylphthalate	DIBP	84-69-5	278	4,11	
Dibutylphthalate	DBP	84-74-2	278	4,57	
Dimethoxyethylphthalate	DMEP	117-82-8	282	2,90	
Diisopentylphthalate	DIPP	605-50-5	306	5,60	
Dipentylphthlate	DPP	131-18-0	306	4,46	
Benzylbutylphthalate	BBP	85-68-7	312	4,70	

Tabla I.7. Continuación

Nombre común	Abreviatura	CAS	Pm	log K_{ow}	Estructura
Ftalatos					
Diisoheptylphthalate	DIHP	41451-28-9	362	7,41	
Di-2(ethylhexyl)phthalate	DEHP	117-81-7	390	7,73	
Dicyclohexylphthalate	DCHP	84-61-7	330	6,20	
Diphenylphthalate	DPhP	84-62-8	318	4,10	
Di-n-octylphthalate	DNOP	117-84-0	390	8,30	
Diisononylphthalate	DINP	28553-12-0	419	8-10	
Diisodecylphthalate	DIDP	89-16-7	446	10	
Adipatos					
Dimethyladipate	DMA	627-93-0	174	1,03	
Diethyladipate	DEA	141-28-6	202	2,47	
Di-2(ethylhexyl)adipate	DEHA	103-23-1	370	8,94	

Los ***adipatos*** son ésteres obtenidos a partir del ácido adípico, que actualmente se produce por la mezcla de ciclohexanol y ciclohexanona (llamado "aceite KA", que proviene de la abreviación de "*ketone-alcoho*l"). Este aceite se oxida con ácido nítrico para procesar el ácido adípico[42]. Los adipatos estudiados en este trabajo se muestran en la Tabla **I.7**.

5.2.2. Usos y aplicaciones

Alrededor de un 93% de los plastificantes son ftalatos, debido a su bajo coste de producción, correspondiendo el 7% restante a ésteres o poliésteres basados en adipatos o ácido fosfórico, entre otros. Debido a sus propiedades, rendimiento y bajo coste, tanto ftalatos como adipatos son empleados para otorgar flexibilidad a los materiales. El 87% de los ftalatos se emplean para ***fabricar cloruro de polivinilo blando*** (PVC blando), mientras que el 13% restante se usa para la fabricación de barnices, lacas, insecticidas…

Los ftalatos usados en la industria tienen cadenas alquílicas entre 1-13 átomos de carbono y es esta diferencia en el número de átomos de carbono lo que les confiere diferentes propiedades y, por lo tanto su uso en diversos ámbitos. Los de bajo peso molecular, cuyas cadenas alquílicas son inferiores a 6 átomos de carbono, no se suelen emplear solos como plastificantes debido a su alta volatilidad pero, combinados con otros tienen un amplio uso en la fabricación de cosméticos, repelentes de insectos, tintas, lacas o adhesivos, mientras que los de elevado peso molecular (cadenas entre 7 y 13 átomos de carbono) se añaden durante la fabricación del PVC, dando lugar a productos versátiles y duraderos (los más empleados para este fin son el DEHP, DIDP y DINP por su capacidad para proporcionar flexibilidad y maleabilidad al plástico). El DEHP representa alrededor del 50% del consumo total europeo[42,43].

En cuanto a su uso como ingredientes en la ***formulación de productos cosméticos***, ftalatos y adipatos tienen múltiples usos como: disolventes y diluyentes sin olor (lacas de uñas, quitaesmaltes, lacas para el pelo), fijadores y disolventes de muchas fragancias, aditivos para aumentar la suavidad y favorecer la penetración cutánea (lociones hidratantes), potenciadores del brillo (esmaltes de uñas), agentes antiespumantes (aerosoles)…

5.2.3. Distribución en el medio ambiente

La liberación de estos compuestos al medio ambiente puede ocurrir durante su producción, o durante la manufactura de los materiales plásticos que los contienen. La mayor parte de los ftalatos presentes en el medio ambiente son consecuencia de liberaciones lentas desde superficies plásticas debido a la acción de agentes atmosféricos o durante la fabricación de las mismas. Debido a esta movilidad desde los productos comerciales, su gran volumen de producción y consumo y el amplio espectro de aplicaciones que presentan, los ftalatos se encuentran ampliamente distribuidos en los diferentes medios de la biosfera y son considerados ***contaminantes ubicuos***.

Como consecuencia de la falta de unión covalente entre los ftalatos y el material polimérico y bajo condiciones de superficie de exposición alta y temperaturas elevadas, los ésteres pueden difundir desde la superficie sólida al aire, a pesar de su baja presión de vapor[44,45]. Aunque los ftalatos en si mismos presentan una ***escasa movilidad en suelos***, filtraciones acuosas de vertederos pueden contener cantidades traza de productos de degradación de los mismos que sean más solubles que los propios compuestos en sí[46]. Una vez que los ftalatos se han liberado al

medio ambiente, su degradación puede ocurrir mediante: hidrólisis, fotodegradación y/o biodegradación, siendo esta última la vía más eficaz, especialmente para eliminar los que llegan a las plantas de tratamientos de aguas residuales o que se encuentran en aguas superficiales, sedimentos o suelos[47].

Los de alto peso molecular presentan una fuerte tendencia a adsorberse en suelos y sedimentos ya que su solubilidad en agua es muy baja (los valores de log K_{OW}, Tabla I.7, indican que su carácter lipofílico aumenta con la longitud de la cadena carbonada). Los ftalatos son degradados por un amplio rango de bacterias y actinomicetos tanto en condiciones aerobias, como anaerobias[48]. La velocidad de ***biodegradación*** depende de la longitud de la cadena alquílica y de la ramificación de la misma, así como de la temperatura de incubación. El DEHP es el contaminante más persistente y estable, con un 75-90% de permanencia en el suelo, después de 6 meses de incubación a temperatura ambiente. Se han realizado también estudios de biodegradación a temperatura ambiente en condiciones anaeróbicas para DMP, DBP y DNOP; los dos primeros se degradan muy rápidamente (más del 90% se elimina entre 4-7 días), mientras que el 80% del DNOP permanece tras una semana de incubación[49-52].

5.2.4. Efectos sobre la salud

Dada su alta tasa de utilización en productos de consumo diario, la exposición humana a los ftalatos puede ocurrir por diferentes vías: ***ingestión,*** debida a la migración de dichas sustancias presentes en el empaquetado de productos alimenticios, ***inhalación*** de los ftalatos presentes en el aire, ***absorción*** a través de la piel (ruta de exposición más significativa cuando se emplean productos cosméticos), o por ***vía intravenosa*** o parenteral en pacientes bajo tratamiento médico que suponga el uso de dispositivos médicos de PVC. Por otra parte, grupos específicos de la población como trabajadores de la industria plástica o pacientes sometidos a diálisis, están expuestos a una mayor concentración de estos compuestos[53-57].

La evaluación toxicológica de estos compuestos ha demostrado que algunos de los ftalatos estudiados son ***disruptores endocrinos***. En 2013, la Organización Mundial de salud (OMS) incluyó ***DBP, BBP y DEHP*** en una lista junto a otras 45 sustancias clasificadas como posibles disruptores endocrinos[58] y cada vez son más los estudios científicos que los califican como tal[59-61]. Asimismo, en diciembre de 2014 la Agencia Europea de Sustancias y Mezclas Químicas (*European Chemicals Agency*, *ECHA*) actualizó una lista con 161 "sustancias que suscitan especial preocupación" entre las que se encuentran ***DEHP, DPP, DIPP, DMEP, DIBP, BBP y DBP***, todos ellos catalogados como tóxicos para la reproducción (Categoría 1B). Resulta preocupante la detección de estos compuestos y/o sus metabolitos en orina, sangre e incluso en el fluido amniótico o en leche materna[62-65], ya que de esta forma, los recién nacidos estarían expuestos a estos compuestos incluso desde antes de nacer; una exposición tan temprana a estas sustancias puede ocasionar daños en el desarrollo de los órganos sexuales, pubertad precoz, disminución de la fertilidad, hiperactividad…[66-68]

5.2.5. Regulación en productos cosméticos

La ***Unión Europea prohibió*** en 2009 el uso de ***DBP, DEHP, DMEP, DPP, DIPP y BBP*** en productos cosméticos, mientras que los demás ftalatos y adipatos estudiados no presentan ningún tipo de restricción en términos de máxima concentración permitida[1].

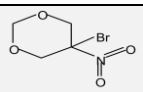
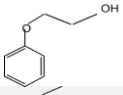
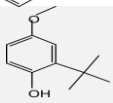
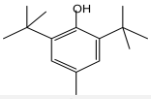
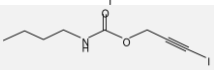
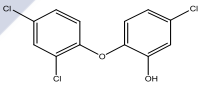
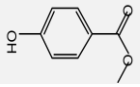
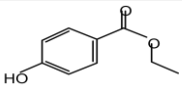
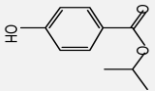
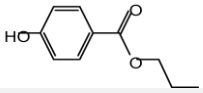
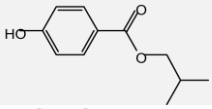
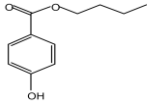
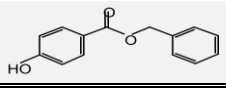
5.3. CONSERVANTES

5.3.1. Introducción y clasificación

Los conservantes son un grupo de ***sustancias antimicrobianas*** que se adicionan a un amplio número de productos (alimentos, preparados farmacéuticos, cosméticos…) para evitar la alteración y degradación de su formulación debido a una posible contaminación debida al crecimiento microbiano. Además, ya que existen muchos productos susceptibles de alterarse e incluso descomponerse en contacto con el oxígeno, muchos de ellos tienen función antioxidante.

Los conservantes objeto de estudio, junto con sus estructuras se presentan en la Tabla I.8.

Tabla I.8. Nombres, CAS, propiedades físicas y estructura de los conservantes estudiados

Nombre común	Abreviatura	CAS	Pm	log K_{OW}	Estructura
Bronidox	BDX	30007-47-7	212	0,25	
Phenoxyethanol	PhEtOH	122-99-6	138	1,1	
Butylhydroxyanisole	BHA	121-00-6	180	3,5	
Butylhydroxytoluene	BHT	128-37-0	220	5,1	
Iodopropynilbutylcarbamate	IPBC	55406-53-6	281	2,4	
Triclosan	TCS	3380-34-5	290	4,8	
Methylparaben	MeP	99-76-3	152	1,9	
Ethylparaben	EtP	120-47-8	166	2,3	
Isopropylparaben	iPrP	4191-73-5	180	2,9	
Propylparaben	PrP	94-13-3	180	2,9	
Isobutylparaben	iBuP	4247-02-3	194	3,4	
Butylparaben	BuP	94-26-8	194	3,5	
Benzylparaben	BzP	94-18-8	228	3,6	

5.3.2. Usos y aplicaciones

Como ya se ha comentado, los conservantes forman parte de la composición de numerosos ***productos de consumo masivo*** como medicamentos, alimentos o bebidas y en menor medida se pueden encontrar en cigarrillos, barnices, pegamentos…

En la ***industria cosmética***, los conservantes son imprescindibles ya que algunos de los factores que influyen en el crecimiento microbiano son la presencia de agua (muchos de los cosméticos presentan una base acuosa), la temperatura de almacenamiento (entre 20-25ºC comienzan a proliferar hongos y levaduras, mientras que las bacterias lo suelen hacer a partir de 30-37ºC) o el pH, entre otros. Por eso, algunos de los requisitos que debe cumplir un conservante son: ser activo a bajas concentraciones y en un amplio intervalo de pH, ser estable a posibles cambios de temperatura y a un tiempo de almacenamiento prolongado, no provocar cambios organolépticos en el producto final y permanecer estable a lo largo de toda la vida útil del producto.

Los más empleados en cosméticos, por lo menos hasta el momento, han sido los ***parabenos*** (ésteres alquilados del ácido p-hidroxibenzoico) cuya estructura básica se muestra en la Figura I.3. Estos compuestos son estables al aire y resistentes a la hidrólisis en agua. Su solubilidad disminuye a medida que aumenta su cadena hidrocarbonada.

Se calcula que el ***75-90% de los cosméticos contienen parabenos*** en concentraciones entre 0,01-0,3% debido a su bajo coste de producción y amplio espectro de actividad antimicrobiana, ya que estos compuestos son muy activos frente a las bacterias Gram positivas, hongos y levaduras. Fueron empleados por primera vez en 1920 en preparados farmacéuticos para evitar la degradación del principio activo. *Methyl-, ethyl-, propyl- y butylparaben* (MeP, EtP, PrP, BuP) son los más encontrados en la formulación de cosméticos, aunque se suelen emplear combinados entre ellos o con otros conservantes para conseguir un efecto sinérgico[69].

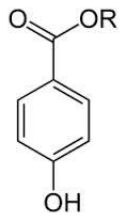


Figura I.3. Estructura básica de los parabenos

5.3.3. Distribución en el medio ambiente

Las ***aguas residuales urbanas*** son el destino principal de los conservantes en el medio ambiente, ya que debido a su uso en productos de consumo masivo, especialmente en cosméticos y productos de cuidado del hogar que se desechan a través del desagüe, una gran cantidad de estas sustancias van a parar a las aguas. En el caso de los parabenos, MeP y PrP son los más abundantes en este tipo de aguas, mientras que EtP, BuP e iBuP también son detectados, normalmente en concentraciones inferiores a la parte por millón (ppm) y los recientemente prohibidos en cosméticos, iPrP y BzP también han sido detectados a bajas concentraciones (< 10 $ng \cdot L^{-1}$)[70,71]. Asimismo, los valores del coeficiente de partición octanol-agua (log K_{OW}) sugieren una alta afinidad, especialmente de los parabenos con una cadena alquílica mayor, por la materia orgánica; de esta forma, se han detectado altos niveles de parabenos en ***lodos de depuradoras***, ***sedimentos marinos***, y también en ***aguas superficiales o de consumo***[72-75]. Además de parabenos, se han encontrado otros conservantes en ***suelos agrícolas*** (posiblemente por contaminación a través de aguas de regadío) e incluso en aire interior[76-79].

5.3.4. Efectos sobre la salud

La vía de exposición más importante a los conservantes es por ***absorción*** a través de la piel o por ***inhalación***. En el caso de los ***parabenos***, su exposición estimada por persona es de 77,5 mg/día (50 mg a través de cosméticos y productos de cuidado personal)[69]. Estos compuestos son absorbidos por la piel y se sugiere que su hidrólisis por las carboxilesterasas de la misma puede ser incompleta, ya que tanto los parabenos como sus metabolitos se han encontrado en ***sangre, orina, fluido amniótico y leche materna***[80-85]. La preocupación por estos compuestos comenzó cuando en 2004 se detectaron trazas de los mismos en ***tumores de mama***[86]. A raíz de esto, muchos autores han planteado que existe una relación directa entre la aplicación de cosméticos, especialmente desodorantes y antitranspirantes, con los niveles de estos compuestos en el tejido cancerígeno[87,88]. Aunque los parabenos no son agentes mutagénicos, su ***actividad estrogénica*** se conoce desde 1998 y ha sido validada tanto con estudios *in vitro* como *in vivo*[89,90]. BuP y PrP son los que presentan una mayor actividad y son numerosos los estudios que asocian algunos de ellos con fenómenos de genotoxicidad y alergias[91-96].

En cuanto a los antioxidantes ***BHA y BHT***, cada vez son más los estudios que los clasifican como posibles ***disruptores endocrinos***[97-99]. El principal inconveniente que presenta el compuesto bromado ***bronidox*** es que se puede descomponer liberando agentes nitrosantes que a su vez pueden reaccionar con aminas alifáticas, presentes habitualmente en la formulación de productos de cuidado personal, dando lugar a la formación de ***nitrosaminas carcinógenas***, mientras que el mayor riesgo que presenta el ***triclosa*****n** para la salud es su capacidad para dar lugar, bajo ciertas condiciones a contaminantes prioritarios como clorofenoles, ***dioxinas*** o compuestos policlorados[100,101].

5.3.5. Regulación en productos cosméticos

La legislación que concierne a los conservantes empleados en cosméticos, especialmente la referida a los ***parabenos*** está en continua revisión debido a los efectos comentados en el apartado anterior. Hasta hace pocos meses, la concentración máxima permitida para estos compuestos en productos cosméticos y de cuidado personal era de 0,4% (expresada en ácido) para un solo parabeno y 0,8% en el caso de que se empleasen mezclas de los mismos.

Desde el ***30 de julio de 2015*** se ***prohíbe*** en la ***Unión Europea*** el uso de cinco parabenos (***isopropyl-, isobutyl-, phenyl-, benzyl- y pentylparaben***) en la formulación de cualquier tipo de cosmético. Asimismo, ***desde el 16 de octubre*** de este mismo año ***se prohíbe en la Unión Europea*** la presencia de ***PrP y BuP*** en productos de permanencia destinados a ser aplicados en zonas del cuerpo de ***menores de 3 años*** cubiertas por pañales, mientras que en otros cosméticos y productos de cuidado personal, su ***máxima concentración*** permitida ***en el producto terminado*** no puede superar el ***0,14%*** (expresado en ácido) para uno solo de ellos, y el 0,8% (de ácido) en el caso de sus mezclas, siempre y cuando las concentraciones individuales no superen el 0,14%[102].

Otro de los conservantes más empleado en cosméticos, el ***phenoxyethanol*** (en los últimos años ha ido sustituyendo a los parabenos, especialmente en los productos comercializados con la etiqueta "sin parabenos") tiene actualmente una concentración máxima permitida de un ***1% en el producto terminado***; sin embargo, la Agencia Nacional Francesa de Seguridad de Medicamentos

(*Agence Nationale de Sécurité du Médicament et des Produits de Santé*, *ANSM*), ha propuesto que se prohíba su uso en productos destinados a menores de 3 años y que se reduzca su concentración en otros productos a un 0,4%[103].

Los límites legales referidos al ***triclosan***, también han variado en el último año, ya que el SCCS consideró que la concentración máxima permitida del ***0,3%*** para todo tipo de productos cosméticos no era segura y, por eso ***desde el 30 de julio de 2015*** esta concentración se permite en dentífricos, pastas de dientes, jabones líquidos, geles de ducha, desodorantes, polvos faciales y cremas correctoras, mientras que para colutorios su concentración máxima se ha reducido a un ***0,2%***[102].

En cuanto a las restricciones de los otros conservantes estudiados, el compuesto bromado ***bronidox*** tiene una concentración máxima permitida de un ***0,1%***; ***IPBC*** está completamente ***prohibido*** para su uso en productos destinados a ***menores de 3 años***, mientras que los antioxidantes BHA y BHT no presentan restricciones. En la Tabla I.9 se muestran las prohibiciones y restricciones en términos de máxima concentración permitida para los conservantes estudiados.

Tabla I.9. Prohibiciones y restricciones de los conservantes estudiados en productos cosméticos.

Conservantes	Prohibiciones y restricciones[1,102]
BDX	0,1%[a]
PhEtOH	1%[a]
BHA	n.r
BHT	n.r
IPBC	Prohibido en productos para menores de 3 años, excepto en productos de baño. 0,02%[a] (cosméticos de aclarado) 0,01%[a] (cosméticos de permanencia, pero prohibido en lociones corporales) 0,0075%[a] (desodorantes)
TCS	0,3%[a] (dentífricos, pasta de dientes, jabones líquidos, geles de ducha, desodorantes, polvos faciales y cremas correctoras) 0,2%[a] (colutorios)
MeP[b]	0,4%[a] (individual); 0,8%[a] (mezclas)
EtP[b]	0,4%[a] (individual); 0,8%[a] (mezclas)
iPrP[b,c]	Prohibido
PrP[b,d]	0,14%[a] (individual); 0,8%[a] (mezclas). Prohibido en productos de permanencia destinados a estar en contacto con zonas del cuerpo cubiertas por pañales en menores de 3 años
iBuP[b,c]	Prohibido
BuP[b,d]	0,14%[a] (individual); 0,8%[a] (mezclas) Prohibido en productos de permanencia destinados a estar en contacto con zonas del cuerpo cubiertas por pañales en menores de 3 años
BzP[b,c]	Prohibido

[a] % referido al producto terminado

[b] Concentración expresada en ácido

[c] La prohibición de estos compuestos entrará en vigor el 30 de julio de 2015

[d] La prohibición/restricción de estos compuestos entrará en vigor el 30 de octubre de 2015

n.r: no restringido en términos de máxima concentración permitida

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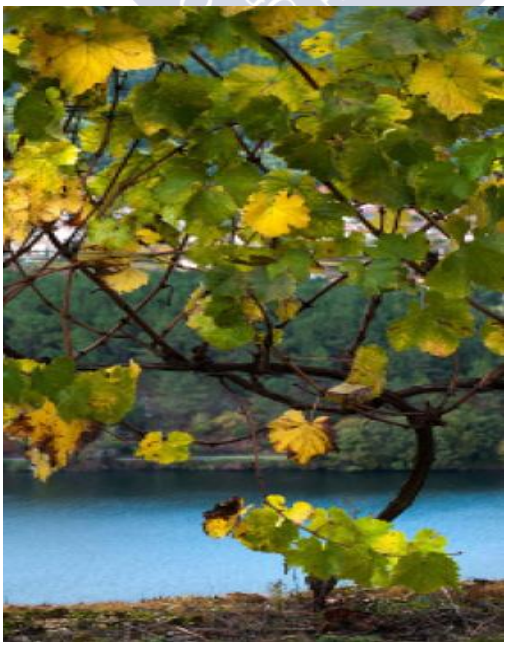
CAPÍTULO II. Fungicidas en vino y subproductos de vinificación



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CAPÍTULO II. FUNGICIDAS EN VINO Y SUBPRODUCTOS DE VINIFICACIÓN

1. OBJETIVOS

El sector vitivinícola gallego ha experimentado en los últimos años un proceso de expansión y desarrollo orientado a obtener vinos de calidad, fomentando las variedades autóctonas. El cultivo de la vid es un pilar fundamental en la agricultura gallega; sin embargo, las condiciones climáticas en esta región favorecen el desarrollo de agentes patógenos, por lo que es necesario la aplicación de tratamientos fitosanitarios para prevenir y/o reducir los daños en los cultivos.

La transferencia tanto de fungicidas como de sus productos de degradación desde los viñedos a los vinos supone un riesgo para la salud humana, ya que muchos de estos compuestos son altamente estables y no son eliminados completamente durante los procesos de vinificación. En Europa, la legislación regula las concentraciones máximas permitidas para fungicidas en diversos alimentos, pero resulta curioso que no exista ningún nivel máximo regulado para vinos o para sub-productos de vinificación tan importantes como el bagazo, que además tiene importantes aplicaciones en el sector farmacéutico o cosmético.

En este Capítulo se pretenden ***desarrollar nuevos métodos de análisis para determinar fungicidas tanto en bagazo como en vinos gallegos***. Las técnicas de extracción empleadas serán PLE y la microextracción-emulsificación asistida por ultrasonidos (USAEME) que han sido seleccionadas en función de la naturaleza de la muestra (sólida o líquida) e intentando cumplir al máximo con los requrimientos de la "Química Verde". La técnica de determinación empleada ha sido GC-MS y, además, para el análisis del bagazo se ha hecho una comparativa entre GC-MS y GC-MS/MS para evaluar las ventajas del modo MS/MS, especialmente en términos de sensibilidad y selectividad.

2. PLAGUICIDAS. DEFINICIÓN Y CLASIFICACIÓN

Según la OMS, se define como *plaguicida* o *pesticida* "todo producto (sustancia o ingrediente activo, así como formulaciones o preparados) destinado a favorecer o regular la producción vegetal, conservarla, y a combatir, eliminar, controlar y prevenir las plagas que puedan afectar a cultivos agrícolas"[104].

Los pesticidas pueden clasificarse en función del organismo sobre el cual ejercen su acción. Los más empleados en agricultura son los herbicidas, seguidos de fungicidas e insecticidas.

Este Capítulo se va a centrar en el estudio de los ***fungicidas***; estos, controlan la actividad de criptógamas (hongos y algunas bacterias) y tienen una importancia especial en el sector vitivinícola, principalmente en zonas con climas adversos para la producción, como es el caso de Galicia, donde existe una mayor proliferación de hongos que pueden causar enfermedades como la podredumbre gris (*Botrytis cinerea*), mildiu (*Plasmopara viticola*) y oidio (*Uncinula necator*)[105]. En la Figura II.1 se muestran los efectos de estas enfermedades de la vid y en la Tabla II.1 su sintomatología y los fungicidas activos frente a cada una de ellas.

Figura II.1. Efectos de podedumbre gris, mildiu y oidio sobre la vid.

Tabla II.1. Fungicidas activos frente a cada enfermedad

Enfermedades	Sintomatología de la enfermedad		Fungicidas activos
	Hojas	**Racimo**	
Podredumbre gris	Manchas grandes pardas en los bordes	Presenta pelaje grisáceo	Iprodione, procymidone, cyprodinil
Mildiu	Manchas color aceite en la parte superior y pelaje en la parte inferior	Presenta pelaje grisáceo y forma de "S"	Metalaxyl, iprovalicarb, benalaxyl, dimethomorph
Oidio	Manchas pequeñas de color ceniza. Los bordes de tuercen hacia arriba	Presenta color ceniza	Myclobutanyl, kresoxim-methyl

En la Tabla II.2 se muestran los fungicidas estudiados, que seleccionados en base a estudios previos que demuestran que son los más habituales en el tratamiento de los viñedos gallegos[106].

Tabla II.2. Nombre, CAS, propiedades físicas y estructura de los fungicidas estudiados.

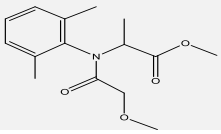
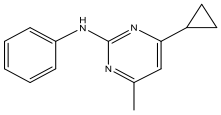
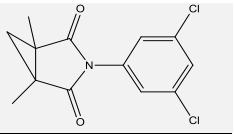
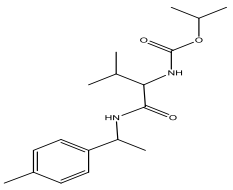
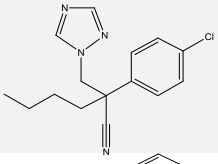
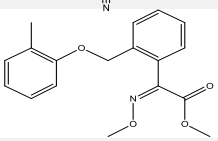
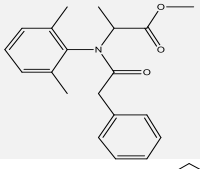
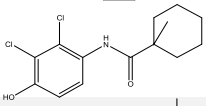
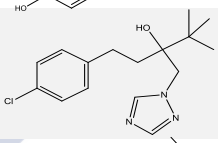
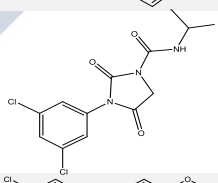
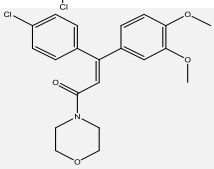
Nombre común	Número CAS	Pm	Log K_{ow}	Punto de ebullición (ºC)	Estructura
Metalaxyl	70630-17-0	279	1,65	394	
Cyprodinil	121552-61-2	225	3,90	406	
Procymidone	32809-16-8	283	3,30	478	

Tabla II.2. Continuación

Nombre común	Número CAS	Pm	Log K_{OW}	Punto de ebullición (ºC)	Estructura
Iprovalicarb	140923-17-7	320	3,20	150[a]	
Myclobutanyl	88671-89-0	288	2,89	465	
Kresoxim-methyl	143390-89-0	313	3,40	429	
Benalaxyl	71626-11-4	325	3,54	468	
Fenhexamide	126833-17-8	301	3,51	230	
Tebuconazole	107534-96-3	307	3,70	350	
Iprodione	36734-19-7	329	3,12	545	
Dimethomorph	110448-70-5	387	2,68	585	

[a] Temperatura de degradación. Se descompone antes del punto de ebullición.

3. USOS Y APLICACIONES DE LOS FUNGICIDAS

Las enfermedades de los cultivos producen un impacto negativo en su rendimiento y calidad, por lo que la prevención y los tratamientos de los agentes que causan dichas enfermedades han de ser un parámetro a controlar exhaustivamente. La mayoría de los fungicidas de uso agrícola se fumigan o espolvorean sobre las semillas, hojas o frutas para impedir la propagación de la enfermedad. Los ***tratamientos frente a los agentes patógenos*** pueden ser ***preventivos*** (previenen la infección y se aplican antes de que los síntomas sean visibles) o ***curativos*** (su objetivo es destruir el hongo ya creado).

Las tres razones principales por las que se emplean fungicidas son:

- Controlar la enfermedad durante el establecimiento y desarrollo de un cultivo.

- Incrementar la productividad de un cultivo y reducir sus daños.

- Mejorar el periodo de almacenamiento de los productos cosechados. Muchas de las pérdidas ocasionadas por enfermedades ocurren después de la cosecha, durante el almacenamiento y algunos hongos que infectan granos producen toxinas (micotoxinas), que pueden afectar gravemente a los seres humanos o animales que los consuman[105].

4. DISTRIBUCIÓN EN EL MEDIO AMBIENTE

La efectividad de los fungicidas depende principalmente de su movilidad, persistencia y transferencia. Deben ser suficientemente móviles como para alcanzar su objetivo y, a su vez persistentes para eliminar el patógeno contra el que actúan. Los fungicidas, en general son moléculas orgánicas con una alta movilidad, de forma que su ***migración a través de suelos***, ***aguas y aire*** resulta sencilla y se puede ver más o menos favorecida en función de sus propiedades físico-químicas (volatilización y solubilización) y de las condiciones ambientales como el viento o la lluvia; de esta forma, regiones con suelos arenosos y altas precipitaciones son más propicios a la lixiviación de los fungicidas, lo que da lugar a que pueda existir contaminación en zonas alejadas del foco de aplicación.

Actualmente, existe una creciente preocupación por la denominada ***contaminación difusa***, principalmente desde parcelas agrícolas, frente a la contaminación puntual[107,108]. La contaminación difusa se define como la introducción de contaminantes, principalmente a un curso de agua superficial o subterránea, a través de vías indirectas; este tipo de contaminación puede ser continua o intermitente, siendo esta última la más común debido a que está relacionada con actividades estacionarias. En el agua, el exceso de nutrientes conduce a una degradación de la calidad del agua, que se acompaña de un aumento de biomasa, la cual a su vez conlleva una mayor turbidez y escasez de oxígeno (hipoxia). Estos procesos tienen consecuencia directa sobre la biodiversidad.

5. EFECTOS SOBRE LA SALUD

En los últimos años, existe una creciente preocupación en cuanto a la ***presencia*** de fungicidas, o de sus productos de degradación ***en alimentos o bebidas*** que puedan ser consumidos por el ser humano, ya que algunos de estos compuestos presentan estructuras químicas muy estables que tardan años en descomponerse a formas menos tóxicas, que se pueden acumular en los tejidos grasos dando lugar a procesos de bioacumulación; es decir, un fungicida que se encuentre en concentraciones muy bajas en el entorno puede concentrarse hasta niveles importantes en tejidos animales. Un estudio reciente realizado sobre vinos embotellados demuestra la presencia de

pesticidas, especialmente fungicidas en el 90% de las muestras analizadas, conteniendo algunos de ellos más de nueve fungicidas distintos[109].

Para distinguir el grado de toxicidad de los fungicidas, es necesario conocer el valor de ***LD_{50}***. Este parámetro, también conocido como ***dosis letal*** se define como: "el estimado estadístico de la cantidad en miligramos de producto tóxico por kilogramo de peso requerida para matar al 50% de una población de ensayo". De forma análoga, se puede definir el ***LC_{50}***, como la concentración de fungicida en aire que puede matar al 50% de una población[110]. En la Tabla II.3 se presentan los valores de LD_{50} estimados para humanos (tanto para exposición oral, como dérmica) y LC_{50}, así como las principales consecuencias de la exposición a estos compuestos.

Tabla II.3. Toxicidad de los fungicidas estudiados[110].

Fungicidas	LD_{50}		LC_{50}	Efectos sobre la salud
	Oral ($mg \cdot Kg^{-1}$)	*Dérmica ($mg \cdot Kg^{-1}$)*	*Inhalación ($mg \cdot L^{-1}$)*	
Metalaxyl	375	>2000	2,29	Irritación de piel y ojos
Cyprodinil	>2000	>2000	>1,20	Irritación del tracto respiratorio, piel y ojos
Procymidone	>5000	>5000	>1,50	Carcinogénico y posible disruptor endocrino.
Iprovalicarb	>5000	>5000	>5,00	Posibles efectos carcinogénicos
Myclobutanyl	1600	>2000	>5,10	Tóxico para el hígado
Kresoxim-methyl	>5000	>2000	>5,60	Irritación de tracto respiratorio, piel y ojos.
Benalaxyl	680	>2000	4,20	No se conocen efectos nocivos para la salud humana
Fenhexamide	>5000	>5000	>5,06	No se conocen efectos nocivos para la salud humana
Tebuconazole	1700	>2000	<5,09	Irritación de ojos y tracto digestivo
Iprodione	>2000	>2500	5,16	Irritación del tracto respiratorio, piel y ojos. Carcinogénico.
Dimethomorph	3900	>2000	>4,42	Graves daños pulmonares por inhalación

6. REGULACIÓN EN VINO Y SUBPRODUCTOS DE VINIFICACIÓN

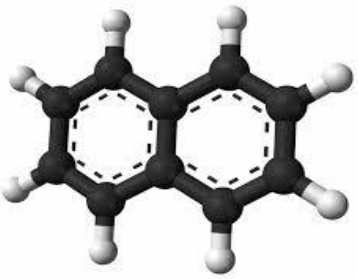
Debido a los posibles efectos adversos de los fungicidas sobre la salud, la Comunidad Europea establece unos ***Límites Máximos de sus Residuos*** (más conocidos como ***MRLs***, *Maximum Residue Levels*) en diversos alimentos como frutas y verduras a través del Reglamento EC No 396/2005 y sus posteriores modificaciones[111]. Sin embargo, llama poderosamente la atención que a día de hoy estos valores estén regulados para uvas (de vinificación y de mesa) y para hojas de parra (Tabla II.4), pero que ***no exista ninguna referencia*** en cuanto a los niveles máximos permitidos ***para vino*** así como para los residuos generados durante su elaboración, como el bagazo. Numerosos estudios han demostrado que muchos de los fungicidas empleados sobre los viñedos no son completamente eliminados durante la elaboración del vino y otras bebidas alcohólicas, y de

hecho se han encontrado en el producto final, con consecuencias negativas no solo para el aroma y la calidad del vino, sino también para la salud de los consumidores[112-117].

Tabla II.4. MRLs permitidos para uvas de vinificación, uvas de mesa y hojas de parra ($mg \cdot kg^{-1}$).

Fungicidas	Uvas de vinificación	Uvas de mesa	Hojas de vid
Metalaxyl	1	2	0,05
Cyprodinil	5	5	0,05
Procymidone	0,01	0,01	0,01
Iprovalicarb	2	2	0,05
Myclobutanyl	1	1	0,02
Kresoxim-methyl	1	1	0,05
Benalaxyl	0,3	0,3	0,05
Fenhexamide	5	5	0,05
Tebuconazole	2	2	0,05
Iprodione	10	10	0,02
Dimethomorph	3	3	0,01

CAPÍTULO III. Hidrocarburos aromáticos policíclicos en superficies de juego y aceite de oliva



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CAPÍTULO III. HIDROCARBUROS AROMÁTICOS POLICÍCLICOS EN SUPERFICIES DE JUEGO Y ACEITE DE OLIVA

1. OBJETIVOS

Los hidrocarburos aromáticos policíclicos, más conocidos por sus siglas en inglés como ***PAHs*** (*polycyclic aromatic hydrocarbons*) están considerados como contaminantes prioritarios debido a sus efectos nocivos sobre la salud. En este Capítulo se va a determinar mediante ***extracción asistida por ultrasonidos*** (ultrasound assisted extraction, UAE) y ***microextracción en fase sólida*** (solid-*phase microextraction, SPME*) seguidas de GC-MS, su presencia en superficies de caucho empleadas en parques infantiles, así como la transferencia de estos compuestos desde las propias superficies al aire y a las aguas de lavado.

Además, se llevará a cabo la optimización de los parámetros experimentales para determinar estos compuestos en aceite de oliva empleando como técnica de extracción una variante de la clásica SPME, que consiste en realizar la extracción bajo condiciones de vacío (Vac-SPME).

2. HIDROCARBUROS AROMÁTICOS POLICÍCLICOS. DEFINICIÓN Y CLASIFICACIÓN

Los hidrocarburos aromáticos policíclicos son un grupo de más de 100 compuestos orgánicos, ya que existen una elevada cantidad de isómeros, formados por carbono e hidrógeno con dos o más anillos de benceno fusionados entre sí. Debido a su ubicuidad, toxicidad tanto teratogénica, mutagénica como carcinogénica y a su alta estabilidad en el medio ambiente, la Agencia Americana de Protección Medioambiental (*Environmental Protection Agency, EPA*) clasificó ya en la década de los 80, 16 de ellos como ***contaminantes prioritarios***. En la Tabla III.1 se muestran algunas de sus propiedades físicas, así como sus estructuras.

Tabla III.1. Nombres, CAS, propiedades físicas y estructuras de los PAHs estudiados.

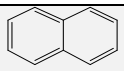
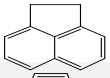
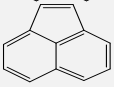
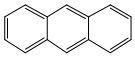
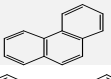
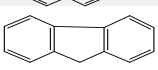
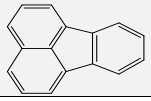
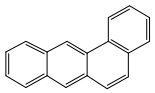
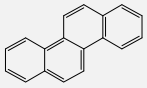
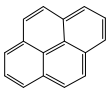
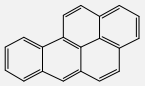
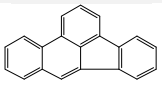
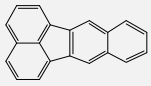
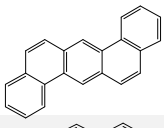
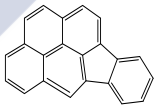
Compuesto	Abreviatura	CAS	Pm	log K_{OW}	Estructura
Naphthalene	NAP	91-20-3	128,2	3,37	
Acenapthene	ACE	83-32-9	154,2	4,33	
Acenapthylene	ACY	208-96-8	152,2	4,07	
Anthracene	ANC	120-12-7	178,2	4,45	
Phenanthrene	PHN	85-01-8	178,2	4,46	
Fluorene	FLU	86-73-7	166,2	4,18	
Fluoranthene	FLA	206-44-0	202,3	5,33	

Tabla III.1. Continuación

Compuesto	Abreviatura	CAS	Pm	log K_{ow}	Estructura
Benzo(a)anthracene	B(a)A	56-55-3	228,3	5,61	
Chrysene	CHY	218-01-9	228,3	5,86	
Pyrene	PYR	129-00-0	202,3	5,32	
Benzo(a)pyrene	B(a)P	50-32-8	252,3	6,04	
Benzo(b)fluoranthene	B(b)F	205-99-2	252,3	6,57	
Benzo(k)fluoranthene	B(k)F	207-08-9	252,3	6,84	
Dibenz(a,h)anthracene	D(ah)A	53-70-3	278,3	6,75	
Benzo(g,h,i)perylene	B(ghi)P	191-24-2	276,3	7,23	
Indeno[1,2,3-cd]pyrene	IND	193-39-5	276,3	7,66	

Se consideran PAHs de bajo peso molecular los que contienen entre 1-3 anillos aromáticos, mientras que los que poseen más de 4, están considerados como de alto peso molecular. Los más perjudiciales para la salud humana son estos últimos, ya que al tener una baja solubilidad en agua son relativamente inmóviles y tienen más tendencia a adsorberse en superficies, así como a acumularse en la cadena trófica.

Los PAHs se originan debido a la combustión incompleta a altas temperaturas (500-800ºC) o por el sometimiento de material orgánico a bajas temperaturas (100-300ºC) durante largos periodos de tiempo. Las ***fuentes de PAHs en el medio ambiente*** pueden ser tanto ***naturales*** (incendios forestales, filtraciones naturales de petróleo, erupciones volcánicas…) como ***antropogénicas*** (emisiones de vehículos, aviones y embarcaciones, procesos industriales, incineración de residuos sólidos urbanos…).

3. DISTRIBUCIÓN EN EL MEDIO AMBIENTE

Al ser unos ***contaminantes*** tan ***ubicuos***, se han encontrado ***en el aire***, tanto en forma de vapores como adheridos a la superficie de pequeñas partículas sólidas (mayores concentraciones en zonas urbanas que rurales), depositados en ***sedimentos***, ***lodos*** o partículas sólidas en el fondo de ríos o lagos, en ***aguas potables***, o en ***aguas superficiales*** desde donde pueden ser transferidos a la atmósfera por volatilización. También se han encontrado en suelos, adheridos a materia orgánica o contaminando aguas subterráneas a través de la lixiviación de los mismos. En el aire, los PAHs pueden degradarse a productos más estables al reaccionar con la luz solar (fenómenos de fotooxidación)[118-121]. Su persistencia en el medio aumenta al incrementarse su peso molecular y su degradación natural no es fácil, ya que depende en gran medida de las condiciones ambientales. Por ejemplo la vida media de una molécula de phenanthrene (PHN) en suelos y sedimentos puede variar entre 16-126 días, mientras que la de benzo(a)pyrene (B(a)P) puede estar entre 229-1400 días[122].

La ***degradación*** más importante y efectiva es la ***microbiana***, que transforma los compuestos en metabolitos menos complejos al desestabilizar el anillo aromático mediante la introducción de dos grupos hidroxilo y, a través de mineralizaciones en formas inorgánicas, CO_2, H_2O (degradaciones aerobias) o CH_4 (procesos anaerobios). La velocidad de la degradación depende también del pH, temperatura, nivel de oxígeno y población microbiana. Algunas de las bacterias más empleadas para este fin son las Pseudonomas aeruginosa, Pseudonomas fluoresens o Mycobacterium spp., que consiguen degradar entre 70-100% de algunos de los PAHs más ligeros presentes en suelos en 40 días[123,124].

4. EFECTOS SOBRE LA SALUD

Al estar presentes en el humo del tabaco, productos de madera tratados con creosota, aire ambiental… la ***vía de exposición más frecuente*** a los PAHs se produce ***por inhalación***; además, alimentos cultivados en suelos contaminados (cereales, verduras, frutas…) pueden contener PAHs y cocinar carne u otros alimentos a altas temperaturas incrementa su concentración en los mismos.

De los 16 clasificados por la EPA como prioritarios, siete (***B(a)A, CHY, B(a)P, B(b)F, B(k)F, D(ah)A e IND***) están considerados como ***posibles carcinogénicos*** en humanos. El B(a)P es sin duda el PAH más estudiado y el único para el cual su carcinogenidad por inhalación está demostrada; además diversos estudios sobre animales han demostrado que la exposición oral a este compuesto induce toxicidad reproductiva, disminuyendo la fertilidad[125-128].

5. REGULACIÓN DE LOS HIDROCARBUROS AROMÁTICOS POLICÍCLICOS

Debido a sus efectos perjudiciales sobre la salud, estos compuestos se encuentran regulados en numerosos productos: ***en alimentos y aceites*** a través del Reglamento (UE) 835/2011, con valores máximos permitidos para B(a)P, B(a)A, B(b)F y CHY[129], ***en aguas superficiales*** a través de la

Directiva 2008/105/CE y sus posteriores modificaciones del Parlamento Europeo y del Consejo, de 16 de diciembre de 2008, que establece las normas de calidad ambiental relativas a la presencia en dichas aguas de sustancias identificadas como prioritarias. La Directiva 2000/60/CE establece 33 sustancias prioritarias, entre las que se encuentran los PAHs, estableciendo valores máximos para B(a)P, B(b)F, B(k)F, B(ghi)P e IND[130]. También ***en aguas destinadas al consumo humano***, se establecen unas concentraciones máximas, a través de la Directiva 98/83/CE del Consejo de 3 de noviembre de 1998, relativa a la calidad de las aguas destinadas al consumo humano y sus posteriores modificaciones[131].

6. HIDROCARBUROS AROMÁTICOS POLICÍCLICOS EN SUPERFICIES DE CAUCHO RECICLADO

Cada vez es más habitual encontrarse con parques, guarderías, campos de fútbol o ***zonas de juego infantiles***, tanto exteriores como interiores ***fabricadas con suelos de caucho*** debido a sus propiedades antideslizantes que tratan de evitar que los más pequeños se hagan daño; muchos de ellos además, presentan colores vistosos o formas divertidas que los hacen más atrayentes para los niños. En la Figura III.1 se ven algunos ejemplos del empleo de suelos de caucho en parques infantiles.

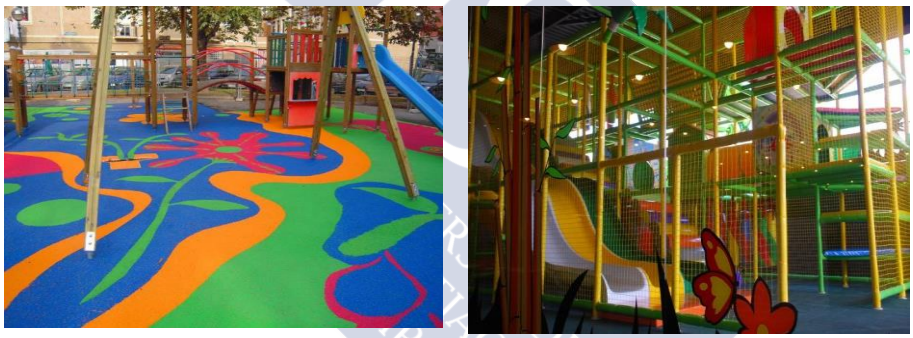


Figura III.1. Suelos de caucho reciclado en parques infantiles (exterior e interior)

El principal componente de estos suelos es el caucho procedente de ***neumáticos reciclados***. ***En España***, desde el años 2006 se prohíbe el almacenamiento de neumáticos usados en vertederos, así como toda clase de incineración sin valoración energética de los mismos y, aunque según la legislación española los neumáticos fuera de uso ***se consideran residuos no peligrosos***[132], en los últimos años numerosos estudios han demostrado la ***presencia de metales y contaminantes orgánicos***, incluyendo PAHs, plastificantes, antioxidantes o antiozonantes en estas superficies[133-136]; además, en el caso de los parques infantiles o campos de fútbol situados al aire libre, el agua de lluvia se puede acumular sobre ellas y arrastrar estos contaminantes hacia suelos o aguas superficiales[137-139]. Asimismo, los situados en el interior de edificios son lavados con agua y detergentes, por lo que estos compuestos tóxicos pueden incorporarse fácilmente en las aguas de alcantarillado.

7. HIDROCARBUROS AROMÁTICOS POLICÍCLICOS EN ACEITE DE OLIVA

En este apartado se incluye el trabajo de investigación realizado durante la breve estancia llevada a cabo en el *Laboratory of Aquatic Chemistry, Department of Environmental Engineering (Technical University of Crete)*. Este trabajo consistió en la optimización de las condiciones experimentales para la ***determinación de PAHs en aceite de oliva*** mediante SPME con vacío (Vac-SPME).

Grecia es el ***tercer país productor de aceite de oliva*** a escala mundial, seguido de España e Italia y los griegos son actualmente los mayores consumidores de aceite de oliva virgen de la Unión Europea, con un consumo medio anual de 21 kg. por habitante. El cultivo de olivos se lleva a cabo en 50 de los 54 municipios el país, destacando la isla de Creta (conocida como la "Isla de los olivos") debido a las excelentes condiciones climáticas que presenta para ello.

Estos compuestos, especialmente los de menor peso molecular se han detectado en aceites de oliva, en algunos casos en concentraciones elevadas[140-142], por lo que la Unión Europea a través del Reglamento EC No 835/2011 establece las ***concentraciones máximas permitidas*** de PAHs ***en aceite de oliva*** (Tabla III.2).

Tabla III.2. Máxima concentración permitida de PAHs en aceites de oliva

Compuestos	**Máxima concentración permitida (μg kg^{-1})**
B(a)P	2,0
Suma de B(aP), B(a)A, B(b)F y CHY	10,0*

*España, Grecia, Italia y Portugal establecen un máximo de 5 μg kg^{-1}.

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CAPÍTULO IV. Técnicas de preparación de muestra y análisis cromatográfico

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CAPÍTULO IV. TÉCNICAS DE PREPARACIÓN DE MUESTRA Y ANÁLISIS CROMATOGRÁFICO

1. OBJETIVOS

La etapa de preparación de muestra es ***fundamental durante el proceso analítico***, ya que de la decisión de cómo tratar la muestra dependerá todo el desarrollo de la metodología analítica. El principal objetivo de esta etapa es la de aislar los compuestos de interés de la matriz (ya sea sólida o líquida), concentrarlos (muchos de ellos se encuentran en concentraciones muy bajas) y disolverlos en un medio compatible con la técnica analítica que se emplee para la determinación de los mismos.

Durante los últimos años se ha intentado que estas técnicas de extracción sean lo más respetuosas posible con el medio ambiente, y que cumplan con los principios de "***Química Verde***"[143]. Por ello, durante el desarrollo de esta Tesis Doctoral se han empleado técnicas que consumen una cantidad mínima de disolventes en comparación con las técnicas clásicas de extracción.

Las técnicas de extracción empleadas han sido la ***dispersión de matriz en fase sólida*** (*matrix solid-phase dispersion, MSPD*), la ***extracción con líquidos presurizados*** (*pressurized liquid extraction, PLE*), la ***extracción asistida por ultrasonidos*** (*ultrasound assisted extraction, UAE*), la ***microextracción-emulsificación asistida por ultrasonidos*** *(ultrasound-assisted emulsification-microextraction, USAEME)* y la ***microextracción en fase sólida*** (*solid-phase microextraction, SPME*).

2. DISPERSIÓN DE MATRIZ EN FASE SÓLIDA

La dispersión de matriz en fase sólida (*matrix solid-phase dispersion, MSPD*) fue introducida por Barker y colaboradores en 1989 y combina aspectos de varias técnicas analíticas, permitiendo realizar la disrupción y dispersión de la muestra en un soporte sólido, generando un material cromatográfico único. El procedimiento "clásico" consta de 4 etapas muy diferenciadas, que se exponen a continuación:

1. Una muestra líquida, semisólida o sólida se añade en un mortero (vidrio, porcelana, ágata) donde se mezcla con un agente dispersante empleando un pistilo, con el objetivo de conseguir una completa ***disrupción y dispersión de la muestra***. Se suele trabajar con tamaños de muestra de 0,5 g. o superiores. En algunos casos se puede emplear un agente desecante como sulfato sódico anhidro (Na_2SO_4), lo que da lugar a un material finamente divido, pero también lo suficientemente seco para la posterior extracción.

2. Una vez completada la etapa de disrupción y dispersión de la muestra, ***la mezcla*** resultante ***se empaqueta*** en una columna vacía, que normalmente es un cuerpo de jeringa vacío, con una frita en su parte inferior. Una vez empaquetada la muestra y teniendo en cuenta los principios de una buena cromatografía (evitar la formación de canales en la columna y no compactar demasiado el material) se coloca una segunda frita sobre la mezcla para después llevar a cabo la compresión de la misma con el émbolo de una jeringa.

3. En cuanto a la ***etapa de elución***, hay dos posibilidades:

a) Los analitos se quedan retenidos en la columna y las interferencias se eluyen en una etapa de lavado y, a continuación, los analitos se eluyen con un disolvente diferente.

b) Las interferencias de la matriz se retienen selectivamente en la columna, mientras que los analitos se eluyen directamente.

Puesto que toda la muestra se encuentra en la columna, también es posible llevar a cabo eluciones múltiples o secuenciales; esto permite el aislamiento de un solo compuesto, de una clase de compuestos o incluso de varias clases de compuestos de una misma muestra. La mayoría de las eluciones se llevan a cabo por gravedad aunque, en algunos casos, el flujo se inicia aplicando presión en cabeza de columna o colocando las columnas en un sistema de vacío (*vacuum box*).

4. Finalmente se puede llevar a cabo una ***etapa de limpieza adicional*** o, directamente analizar la muestra. También se puede recurrir al uso de co-columnas para obtener un mayor grado de fraccionamiento y limpieza del extracto obtenido.

En la Figura IV.1 se muestran de forma esquemática las etapas explicadas anteriormente.

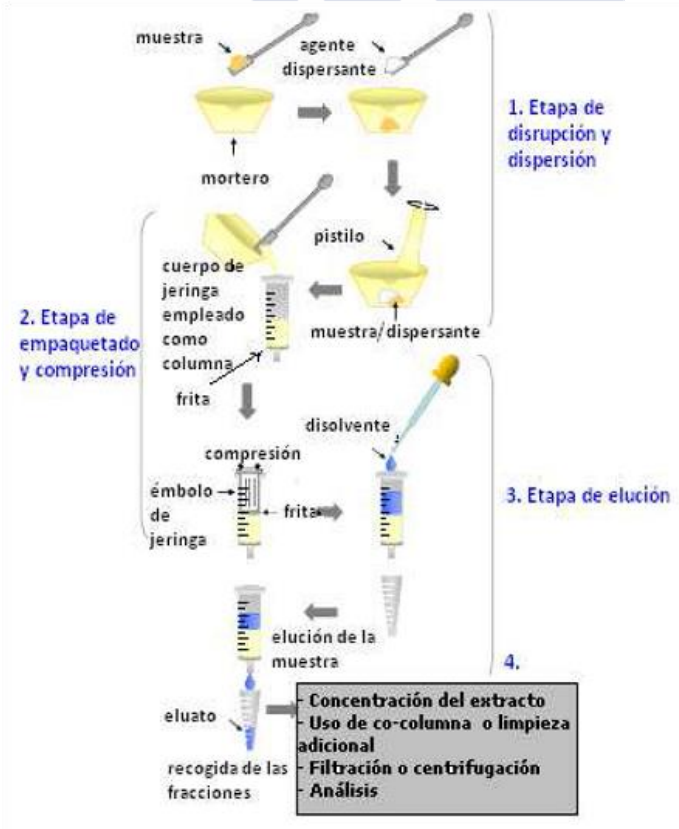

Figura IV.1. Esquema del procedimiento experimental mediante MSPD.

Las ventajas que presenta la MSPD sobre los procedimientos clásicos de tratamiento de muestra son, entre otros:

- El procedimiento analítico se simplifica y acorta drásticamente.

- Se elimina la posibilidad de formación de emulsiones.

- El consumo de disolventes se reduce sustancialmente.

- Se mejora la eficacia de la extracción de los analitos, puesto que toda la muestra se expone al extractante.

2.1. MICRO-DISPERSIÓN DE MATRIZ EN FASE SÓLIDA

Debido a que una de las familias estudiadas en esta Tesis son los ***plastificantes*** (ftalatos y adipatos) que se encuentran presentes ***en*** la mayoría del ***material plástico*** empleado en el laboratorio, uno de los problemas que se planteaba al realizar la MSPD de la forma descrita anteriormente, era el ***riesgo de contaminación*** lo que podía dar lugar a falsos positivos y sobreestimaciones en las concentraciones de las muestras. El riesgo de contaminación por estos compuestos puede estar presente a lo largo de todo el proceso analítico, desde la toma de muestra, hasta el análisis cromatográfico, debido a su ubicuidad, ya que se encuentran presentes en el aire, agua, disolventes orgánicos… por lo tanto, para minimizar su interferencia en el proceso de preparación de muestra mediante MSPD, se decidió ***evitar el material plástico***, sustituyéndolo por vidrio, evitar el uso de guantes de determinados materiales…

Asimismo, el riesgo de contaminación se reduce si el proceso de preparación de muestra es mínimo; esto implica llevarlo a cabo con las mínimas etapas de extracción, mínima concentración de extracto y reduciendo el uso de material y de disolventes.

Teniendo en cuenta todas estas recomendaciones y ya que las muestras cosméticas son homogéneas, lo que permite emplear pequeñas cantidades, se planteó una variación de la tradicional MSPD, dando lugar a una ***MSPD "miniaturizada"*** cuyo esquema se muestra en la Figura IV.2.

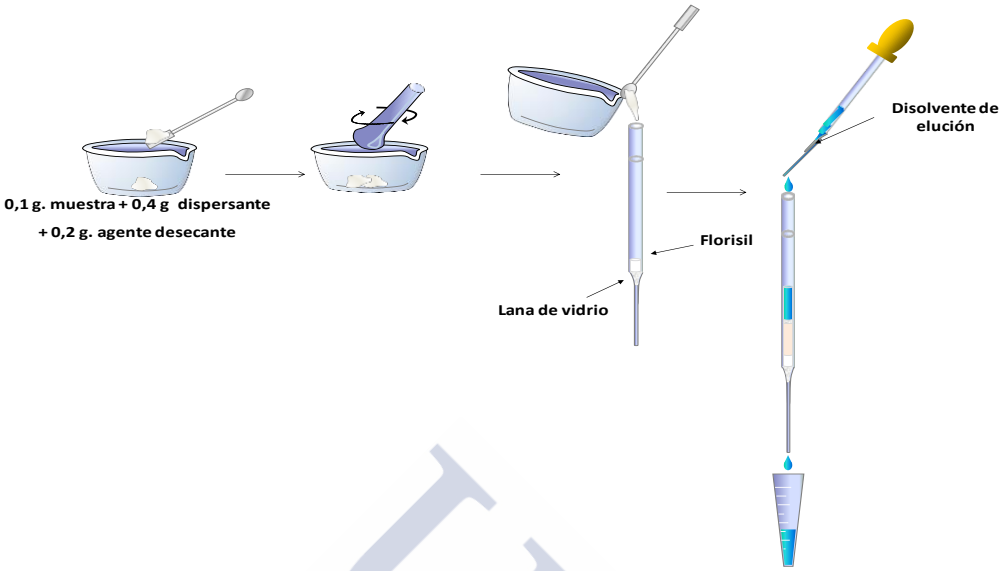

Figura IV.2. Esquema del procedimiento experimental mediante micro-MSPD

Las etapas de esta técnica de microextracción (µ-MSPD) son las siguientes:

1. Se homogenizan en un mortero o vial de vidrio 0,1 g. de muestra, a los que se les añade 0,2 g. de un agente desecante (Na_2SO_4) y 0,4 g. de un agente dispersante (Florisil).

2. Una vez completada la etapa de disrupción y dispersión, la mezcla obtenida se transfiere a una pipeta Pasteur de vidrio (150 mm. de longitud aproximadamente), que contiene en el fondo una pequeña porción de lana de vidrio y 0,1 g. de Florisil (para obtener un mayor fraccionamiento de la muestra y una etapa de limpieza o *clean-up* del extracto obtenido). En la parte superior de la pipeta se coloca otra porción de lana de vidrio y con ayuda de una espátula se compacta la mezcla.

3. Se hace pasar el disolvente correspondiente a través de la columna y se recoge un volumen de extracto (1 o 2 mL), el cual puede ser directamente inyectado en el instrumento cromatográfico, sin necesidad de posteriores etapas de limpieza.

Esta modificación de la clásica MSPD permite que todo el proceso de extracción de los analitos se lleve a cabo en muy poco tiempo y consumiendo muy poca cantidad de muestra, reactivos y disolventes, lo que abarata los costes. Asimismo, se trata de un método muy sencillo ya que todo el material empleado es de uso habitual en cualquier laboratorio.

3. EXTRACCIÓN CON LÍQUIDOS PRESURIZADOS

La extracción con líquidos presurizados (*pressurized liquid extraction, PLE*), también conocida como *extracción con fluídos presurizados* (PFE) o por el nombre comercial *extracción acelerada por disolventes* (ASE^{TM}, Dionex, Sunnyvale, CA, USA) fue introducida por primera vez en 1995. El fundamento de esta técnica se basa en que trabajando a presiones elevadas, los disolventes se encuentran en fase líquida a temperaturas superiores a su punto de ebullición, pero ligeramente inferiores a su punto crítico, lo cual favorece la eficacia de la extracción. Los principales parámetros que influyen en esta técnica son:

- ***Temperatura***: Tiene que ser los suficientemente elevada como para favorecer la cinética de la extracción, pero sin degradar los analitos. Al aumentar la temperatura el disolvente disminuye su viscosidad, por lo que penetra con mayor facilidad en los poros de la matriz, favoreciendo la difusión de los analitos.

-Presión: Debe ser lo suficientemente elevada como para mantener el disolvente en estado líquido. Existen dos modos de llevar a cabo las extracciones. En *modo estático*, en el que el disolvente es introducido en la celda y esta se mantiene a presión constante durante un tiempo determinado; tras esto, la celda se vacía recogiendo todo el extracto obtenido en un vial colector y *modo dinámico*, en el que el disolvente está pasando continuamente un flujo constante a través de la celda presurizada.

Además de temperatura y presión que son los parámetros más críticos, existen otros que pueden afectar a la eficacia de la extracción y que deben tenerse en cuenta a la hora de desarrollar un método PLE, como son: el disolvente empleado, el tiempo de extracción o el número de ciclos.

En la Figura IV.3 se muestra un esquema general del proceso de PLE.

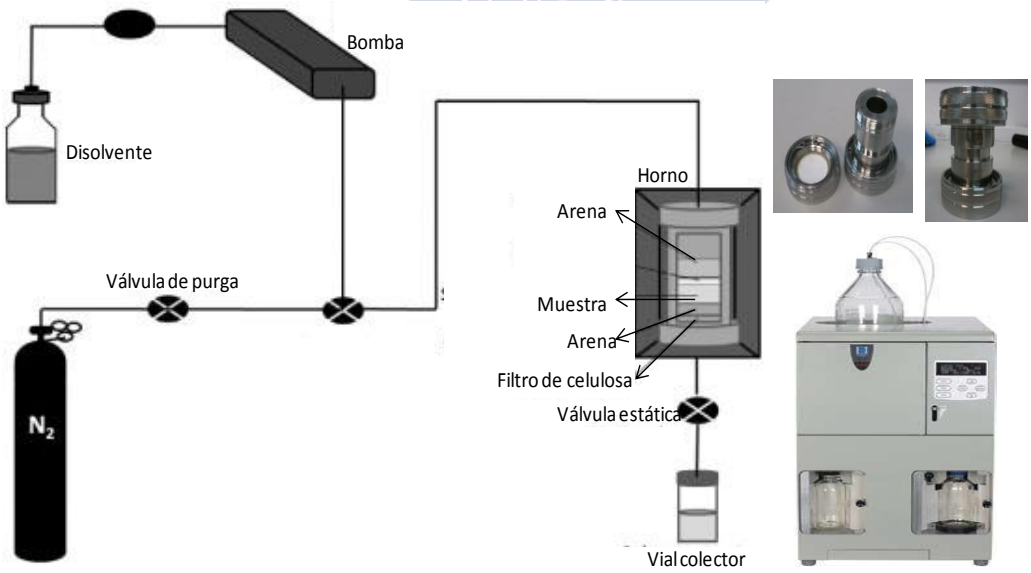

Figura IV.3. Esquema procedimiento PLE, celda de extracción e instrumento empleado.

Las etapas de una extracción mediante PLE se detallan a continuación:

1- Una vez pesada, ***la muestra se coloca en la celda de extracción***. Habitualmente, para evitar que las fritas metálicas situadas en ambos extremos de la celda de extracción se obstruyan, se colocan filtros de celulosa en ambos extremos. Asimismo, para hacer más eficaz la extracción es recomendable llenar el volumen muerto de la celda con un material inerte, habitualmente arena o tierra de diatomeas.

2- Una vez introducida la celda de extracción en el sistema, ocurren las etapas de ***calentamiento, llenado de la celda y extracción***. La celda, conteniendo la muestra, se calienta hasta la temperatura apropiada durante un tiempo de equilibrio (habitualmente 5 minutos). A continuación, el disolvente es introducido en la celda y esta se mantiene a presión constante durante un tiempo determinado a la presión y temperatura seleccionadas.

3- Tras la extracción, el extracto se transfiere a un vial colector mientras que la celda se enjuaga con varias porciones de disolvente nuevo (*flush*). A continuación todo ***el sistema se purga*** con nitrógeno presurizado durante 1-2 minutos.

4- Por último, ***el extracto obtenido puede ser directamente analizado***, aunque dependiendo de la técnica de determinación empleada, puede ser necesaria una etapa de filtración, concentración, derivatización…

4. EXTRACCIÓN ASISTIDA POR ULTRASONIDOS

La extracción asistida por ultrasonidos (*ultrasound assisted extraction, UAE*) es una ***técnica muy sencilla, rápida y de bajo coste*** que emplea los ultrasonidos, ondas acústicas de frecuencia inaudible para el oído humano. Puede ser aplicada tanto a muestras sólidas como líquidas.

El procedimiento consiste en ***aplicar la energía de ultrasonidos*** a un disolvente orgánico, lo que provoca una agitación continua de la muestra en el disolvente, ***facilitando*** así los procesos de transferencia de masa entre ambas fases al existir una ***mayor penetración del disolvente en las*** distintas ***matrices***. Como resultado, se obtienen extracciones muy eficaces en cortos periodos de tiempo.

Los ultrasonidos viajan a través del disolvente en forma de onda y lo hacen de forma alterna provocando contracciones y expansiones de dicho medio. Durante la expansión se produce un aumento negativo de la presión lo que da lugar a la formación de cavidades (fenómeno de cavitación). Esta energía liberada provoca un aumento de la temperatura que facilita la solubilidad de los analitos y este aumento de temperatura, junto con las presiones alcanzadas, provoca una mayor penetración del disolvente en la muestra[144,145].

UAE puede realizarse mediante un “baño de ultrasonidos” o mediante una “sonda de ultrasonidos”. En el primer caso, se aplica sobre un baño de agua una frecuencia de ultrasonidos determinada y constante (habitualmente de unos 40 KHz). El empleado durante este trabajo se

muestra en la Figura IV.4. En el caso de la sonda, ésta puede ser introducida en el interior de la muestra y la frecuencia se puede regular, de forma que se pueden realizar extracciones más enérgicas (para muestras muy complejas).

Figura IV.4. Baño de ultrasonidos empleado en este trabajo

5. MICROEXTRACCIÓN–EMULSIFICACIÓN ASISTIDA POR ULTRASONIDOS

Como ya se ha comentado en el punto anterior, la aplicación de energía de ultrasonidos favorece los procesos de transferencia de masa entre dos fases inmiscibles. Esto, junto con una gran área de contacto entre ambas debido a la formación de una emulsión, lleva a una elevada eficiencia de extracción en un breve periodo de tiempo[145,146].

La microextracción-emulsificación asistida por ultrasonidos (*ultrasound-assisted emulsification-microextraction, USAEME*) fue propuesta por Regueiro y colaboradores en 2008[144] para el análisis de contaminantes emergentes y pesticidas en aguas. Su fundamento se basa en la ***formación de una emulsión de un microvolumen de disolvente orgánico*** (fase dispersa) ***en una matriz acuosa*** (fase continua) ***por acción de ultrasonidos***. Durante este proceso tiene lugar la ***transferencia de los analitos*** desde la muestra acuosa ***a las microgotas de disolvente*** orgánico que se encuentran dispersas en la misma. ***Ambas fases*** son ***separadas*** posteriormente ***mediante centrifugación y el extracto orgánico*** resultante ***es recogido*** para su análisis.

La USAEME es una ***técnica rápida, muy sencilla y económica***, que puede ser aplicada para la extracción de compuestos orgánicos en muestras acuosas. Además es una técnica respetuosa con el medio ambiente ya que los volúmenes de disolvente orgánicos son inferiores a 200 µL.

La extracción se lleva a cabo en el interior de un tubo de vidrio de fondo cónico en que se introduce la muestra acuosa y se adiciona un microvolumen de un disolvente orgánico inmiscible y de densidad superior a la del agua, generalmente halogenado. A continuación el tubo es sometido a ultrasonidos en el interior de un baño de agua, lo que produce la emulsificación del sistema. La disrupción de la emulsificación se consigue por centrifugación y el extracto orgánico sedimentado en el fondo del tubo es recogido con una microjeringa para su análisis. En la Figura IV.5 se muestra un esquema del procedimiento.

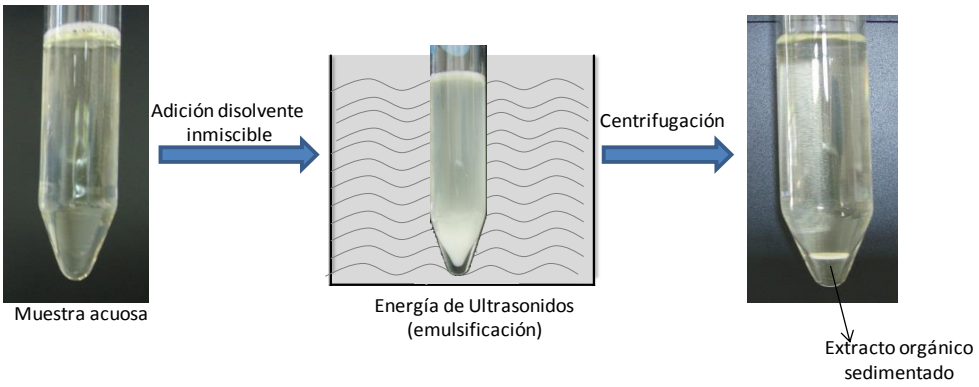

Figura IV.5. Esquema del procedimiento de extracción mediante USAEME.

La eficiencia del proceso de microextracción-emulsificación puede verse afectada por varios factores (disolvente empleado, relación de volúmenes de fases, tiempo de irradiación ultrasónica, fuerza iónica del medio…) que será preciso optimizar para conseguir una buena eficiencia de extracción.

6. MICROEXTRACCIÓN EN FASE SÓLIDA

Más conocida por sus siglas en inglés como SPME, *solid-phase microextraction*, fue propuesta por Pawliszyn en los años 90[147] y se basa en la ***extracción*** de los analitos de la matriz de una muestra ***mediante una fibra de sílice fundida*** (químicamente inerte y estable a altas temperaturas) que está ***recubierta por un sorbente***, en la mayoría de los casos polimérico, ***seguida por la desorción de los analitos mediante temperatura***. Las etapas de ***muestreo, extracción y enriquecimiento*** se realizan ***en un solo paso*** y ***no se necesita el empleo de disolventes orgánicos*** para la desorción de los mismos.

El pequeño tamaño de la fibra y su geometría permiten alojarla en el interior de una aguja hueca de acero inoxidable que la protege antes y después del proceso de extracción; este dispositivo se coloca en un soporte comercial especialmente diseñado (Figura IV.6). Antes de comenzar la extracción el émbolo debe estar retraído y una vez que se ha perforado el septum del vial que contiene la muestra, éste se baja para iniciar el proceso de exposición y extracción de la muestra a la fibra. Se comercializan diferentes tipos de fibras, según la naturaleza del recubrimiento de su material polimérico. Es importante destacar que la eficacia del proceso de extracción dependerá de la constante de distribución entre la fase de la fibra y los analitos de la muestra, por lo que es importate elegir el recubrimiento idóneo según los analitos que se pretendan estudiar[148].

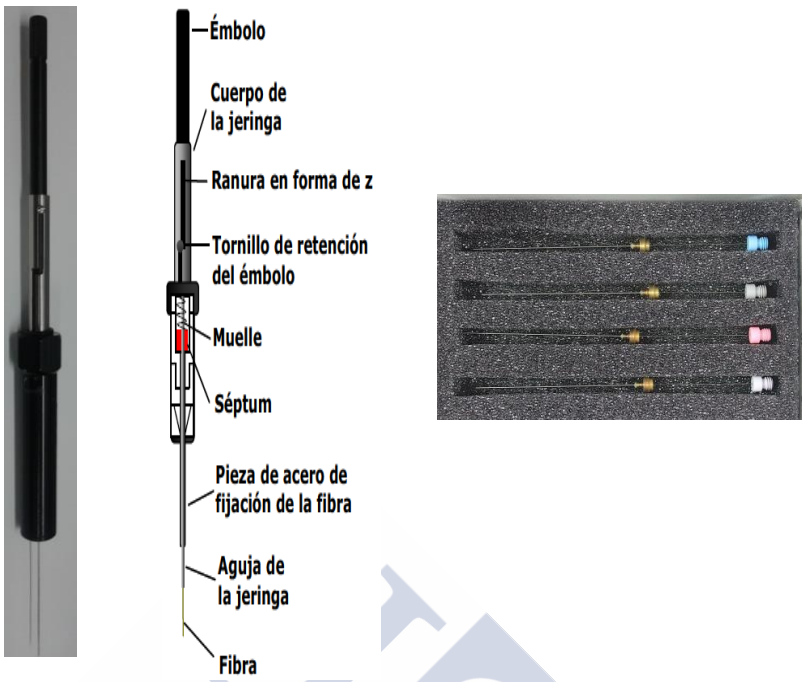

Figura IV.6. Dispositivo comercial de SPME, esquema de sus partes y distintas fibras.

La SPME es una ***técnica rápida, sencilla y fácilmente automatizable*** que se puede ***aplicar tanto a muestra sólidas, como líquidas o gaseosas***. Hay que destacar que la extracción se considera completa cuando se alcanza un equilibrio de distribución entre las distintas fases implicadas. El transporte de los analitos desde la matriz hacia la fibra comienza en el momento en el que la fibra se expone a la misma y esta exposición puede ocurrir de diferentes formas:

a) **Extracción en el espacio de cabeza** del vial por encima de la muestra (**HS-SPME**, *headspace-solid phase microextraction*): Los analitos son extraídos de la fase gaseosa que está en equilibrio con la muestra. En este modo de extracción los analitos se transportan, en primer lugar de la muestra al espacio de cabeza y posteriormente son atrapados en la fibra (figura IV.7a).

b) **Extracción por inmersión directa** (**DI-SPME**, *direct inmersion-solid phase microextraction*). Los analitos se extraen mediante la inmersión de la fibra en la muestra (Figura IV.7b).

c) **Extracción empleando una membrana de protección**: La fibra se protege con una membrana que permite el paso de los analitos pero no de las interferencias. Se emplea para análisis de muestras muy complejas.

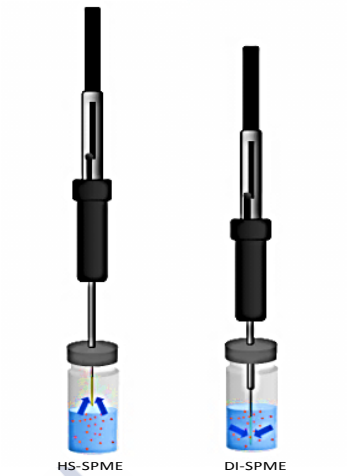

Figura IV.7. a) esquema HS-SPME. **b)** esquema DI-SPME

Las variables que más afectan a la eficacia de la extracción mediante SPME son:

- La elección del ***recubrimiento polimérico***: Dependerá de las polaridades de los analitos de interés.

- ***Tiempo de extracción***: Ya que esta técnica se basa en un proceso de equilibrio, será necesario que la fibra esté expuesta el tiempo necesario para que la extracción sea completa.

- ***Temperatura de extracción***: El incremento de la temperatura favorece la migración de los analitos hacia la fibra y, por lo tanto, reduciendo el tiempo necesario para alcanzar el equilibrio pero por otra parte, la etapa de absorción en la fibra es un proceso exotérmico, por lo que un aumento de la temperatura en condiciones de equilibrio, implica una disminución en la cantidad de analito extraída.

- ***Agitación*** de la muestra: Normalmente incrementa la difusión de los analitos desde la matriz hacia el espacio de cabeza o hacia la fibra, disminuyendo el tiempo de extracción.

- Uso de ***modificadores del medio*** o de matriz: Para favorecer la extracción de los compuestos se puede modificar la fuerza iónica del medio, el pH, o adicionar agua o disolventes orgánicos a muestras sólidas para una mejor liberación de los analitos de la matriz hacia el recubrimiento de la fibra.

6.1. MICROEXTRACCIÓN EN FASE SÓLIDA CON VACÍO

Muchos autores han demostrado un ***aumento en la eficacia de extracción al reducir la presión durante el procedimiento de SPME*** (*Vac-SPME, Vacuum-solid-phase microextraction*)[149,150]. Esta variante de la SPME clásica ha sido ampliamente estudiada por Psillakis y colaboradores[151-153] y ha sido aplicada con éxito para determinar PAHs y ftalatos en muestras ambientales y suelos.

Para el caso de muestras líquidas, la ventaja que presenta esta técnica es que evacuando el aire antes de la introducción de la muestra, se elimina la posibilidad de pérdidas de los compuestos mas volátiles. Hay que destacar que Vac-SPME solo puede ser aplicada a compuestos cuyas constantes de Henry (K_H) estén por debajo o próximas a los valores umbral de $1{,}2\times10^{-5}$ y $1{,}6\times10^{-4}$ atm m^3 mol^{-1} ya que en estos casos la resistencia de la transferencia de masa entre la interfase espacio de cabeza/muestra controla la evaporación, por lo que una reducción en la presión total se traducirá en un proceso más rápido de extracción global. Para compuestos con valores de K_H entre los valores umbral antes mencionados y menores de $5x10^{-3}$ atm m^3 mol^{-1} no se espera que se mejore la extracción, ya que en estos casos, quien controla la eficacia de la extracción es la transferencia de masa en la propia muestra líquida y este proceso es independiente de las condiciones de presión en el espacio de cabeza[151-153].

En la Figura IV.8 se muestra un esquema general del procedimiento de extracción.

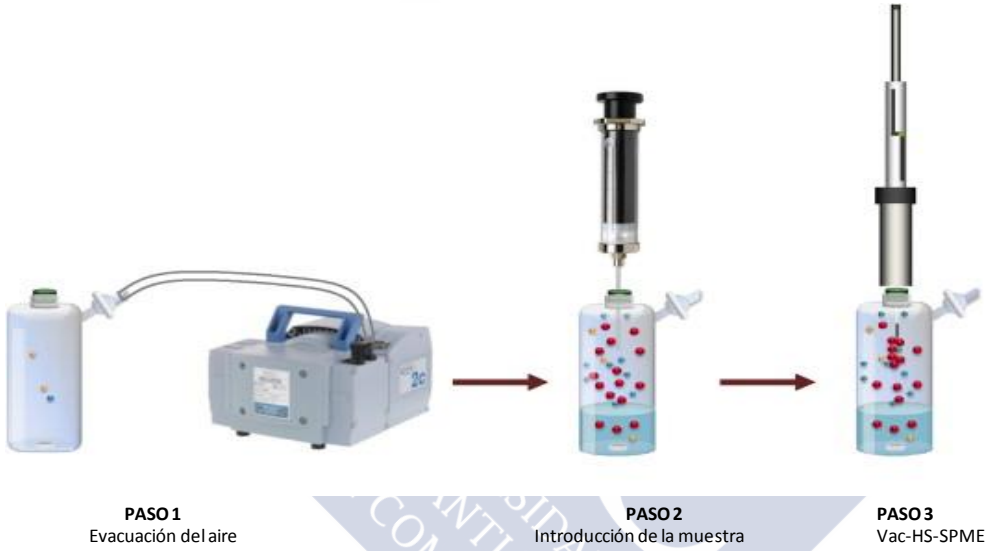

Figura IV.8. Esquema del procedimiento de extracción mediante Vac-HS-SPME

En un primer paso se evacua el aire del interior del vial a través de una bomba de alto vacío. A continuación la muestra líquida es introducida en el vial a través de un septum, con ayuda de una jeringa y se deja un tiempo determinado (normalmente 10 minutos) hasta que se alcance el equilibrio. Por último la fibra de SPME se expone al espacio de cabeza durante el tiempo oportuno.

En este caso, al igual que ocurre con la SPME convencional, las condiciones experimentales de extracción deberán ser optimizadas para obtener la máxima eficacia de extracción.

7. CROMATOGRAFÍA DE GASES-ESPECTROMETRÍA DE MASAS

El análisis de los componentes que forman parte de una muestra compleja como son las analizadas en esta Tesis, es una tarea muy complicada que requiere la utilización de técnicas cromatográficas con detectores selectivos, como la espectrometría de masas (MS).

Se ha seleccionado la ***cromatografía de gases (GC)*** ya que es una técnica que presenta la cualidad de conseguir la separación de compuestos volátiles o semivolátiles térmicamente estables a temperaturas de 350-400ºC, en muestras muy complejas; pero aun así, una vez separados todos los componentes de una mezcla problema, el único dato del que se dispone es el tiempo de retención de los correspondientes picos cromatográficos y este dato no es suficiente para una identificación inequívoca.

Para ello se hace necesaria la ***espectrometría de masas*** ya que como detector presenta ventajas como la capacidad de identificación de forma prácticamente inequívoca, al proporcionar un espectro característico de cada molécula, gran sensibilidad, información estructural y es una técnica rápida: se puede realizar un espectro en décimas de segundo, por lo que se puede monitorizar para obtener información en tiempo real sobre la composición. En la figura IV.9 se muestra un esquema de GC-MS.

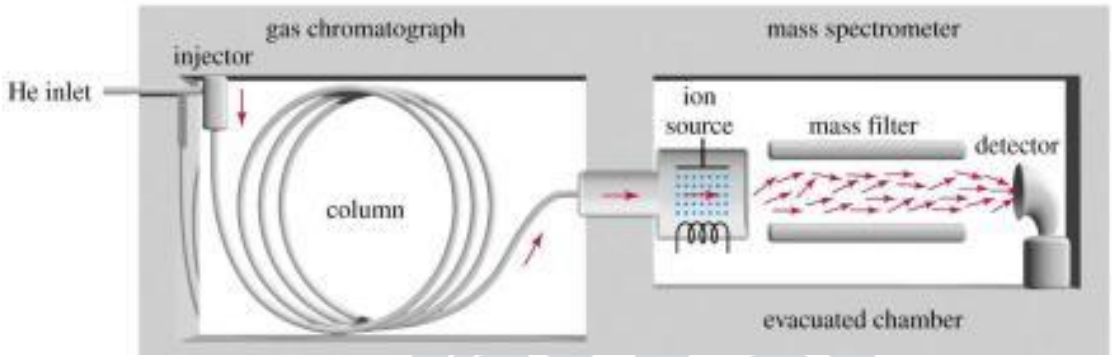

Figura IV.9. Esquema general de un cromatógrafo de gases acoplado a un espectrómetro de masas (GC-MS)

En esta Tesis se ha empleado la ionización por ***impacto electrónico***, en la que las moléculas de la muestra son ionizadas por un haz de electrones de elevada energía y se han utilizado equipos con ***analizadores cuadrupolares (Q)***, muy empleados en hibridación con GC, ya que al no utilizar campos magnéticos para la dispersión, se encuentra libre de los problemas derivados de la histéresis magnética y, por lo tanto, se puede emplear para realizar barridos cromatográficos en tiempo real.

Los distintos ***modos de trabajo en GC-MS*** son los siguientes:

- ***FULL SCAN***: se hace un barrido de todas las masas, comprendidas entre un rango especificado.
- ***SIM*** (*selected ion monitoring*): se monitorizan selectivamente determinados iones, aumentando la selectividad del método al reducir las interferencias.

7.1. CROMATOGRAFÍA DE GASES-ESPECTROMETRÍA DE MASAS EN TÁNDEM

La cromatografía de gases combinada con espectrometría de masas en tándem (GC-MS/MS) permite obtener una mayor selectividad y sensibilidad analítica, minimizando las interferencias de la matriz. En este sentido, el empleo de un triple cuadrupolo (TQ), permite incrementar la especificidad del método. En la Figura IV.10 se muestra el esquema de un triple cuadrupolo. La ventaja de los

espectrómetros de masas en tándem respecto a los cuadrupolos sencillos es que permite seleccionar un ion pseudomolecular (ion padre o precursor) en el primer cuadrupolo (Q1), provocar su fragmentación en la celda de colisión (Q2) y seleccionar el fragmento originado (ion hijo o producto) en el segundo cuadrupolo (Q3). Esta posibilidad se traduce en la ***disminución del ruido de fondo*** respecto al que se produce en un GC-MS simple y, por lo tanto en un ***aumento de la sensibilidad y selectividad***, ya que el fragmento monitorizado en Q3 procederá únicamente del ion padre seleccionado en Q1.

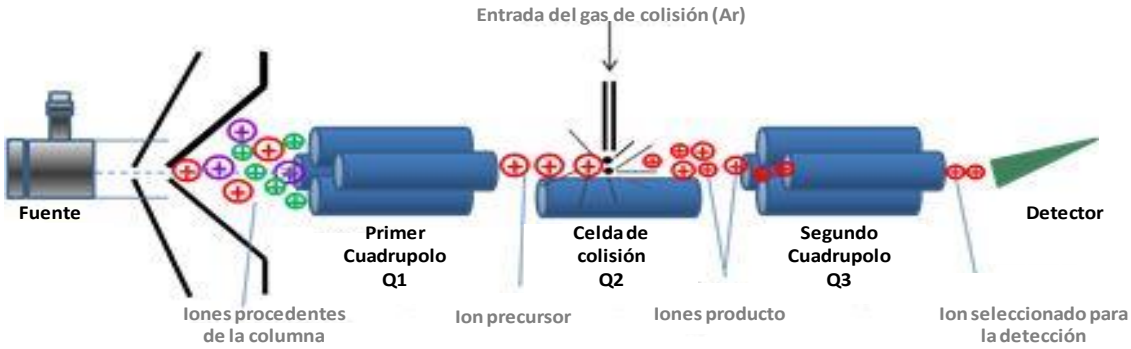

Figura IV.10. Esquema de un analizador de masas de triple cuadrupolo (TQ)

Con la espectrometría de masas en tándem, además de trabajar en los modos Full Scan o SIM, existe un tercer modo de trabajo:

- ***SRM*** (*selected reaction monitoring*): los dos cuadrupolos trabajan en modo SIM; es decir, en Q1 se selecciona una determinada masa/carga, se provoca su fragmentación en Q2 y un determinado ion hijo se monitoriza en Q3. A cada combinación ion padre-ion hijo concreto se le denomina transición. En un método SRM se pueden seleccionar varias transiciones. Este modo se emplea para realizar análisis cuantitativos, aportando la máxima sensibilidad y selectividad en la determinación de compuestos conocidos.

En la Figura IV.11 se muestran los dos instrumentos empleados durante el desarrollo de esta Tesis Doctoral.

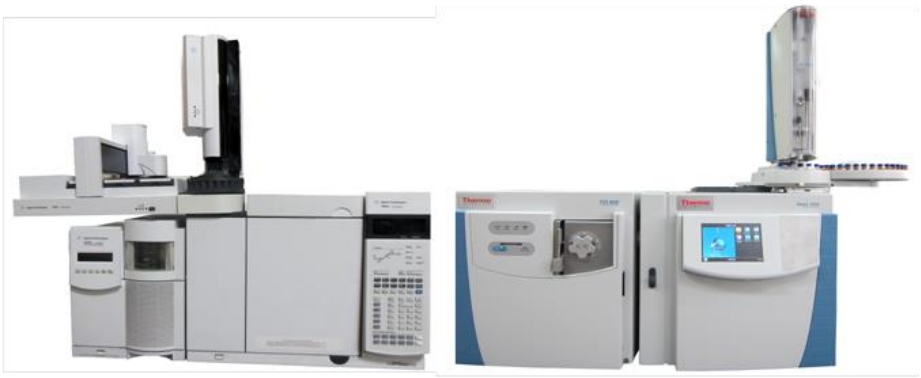

Figura IV.11. Equipos GC-MS y GC-MS/MS empleados durante el desarrollo de esta Tesis

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[1] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast), in Off. J. Eur. Union L342/59., 2009.

[2] Cosmetics Europe. The personal care association (CEAR). Available at: http://www.cosmeticseurope.eu/using-cosmetics-colipa-the-european-cosmetic-cosmetics-association/cosmetics-through-history.html (Accessed March 2015).

[3] Cosmetics Europe. The personal care association (CEAR). Anual report, 2013. Available at: http://www.cosmeticseurope.eu/publications-cosmetics-europe-association/annual-reports.html?view=item&id=98 (Accessed March 2015).

[4] Salud y vida sana. Nueva normativa para el control de los cosméticos. Available at: http://www.saludyvidasana.es/2013/09/nueva-normativa-para-el-control-de-los-cosmeticos/ (Accessed March 2015).

[5] Vida Sana. Artículo: Cosmética natural. Available at: http://www.vidamassana.com/actualidad/articulos/cosmetica-natural/ (Accessed March 2015).

[6] Cosmética natural. Certificados en cosmética natural. Available at: http://www.cosmetica-natural.eu/es/cosmeticos-naturales-certificados/imo-control-vida-sana/ (Accessed March 2015).

[7] U.S. Food and Drug Administration. U.S. Department of Health and human services. Available at: http://www.fda.gov/Cosmetics/default.htm (Accessed March 2015).

[8] Health Canada. Consumer Product Safety. Cosmetic Ingredient Hotlist. Available at: http://www.hc-sc.gc.ca/cps-spc/cosmet-person/hot-list-critique/hotlist-liste-eng.php (Accessed March).

[9] Cosmetic Regulations C.R.C., c. 869 Food and Drugs Act. Available at: http://www.wipo.int/wipolex/es/text.jsp?file_id=207907 (Accessed March 2015).

[10] Pharmaceuticals and Medical Devices Agency. Available at: http://www.pmda.go.jp/english/ (consultado en marzo, 2015).

[11] K. Schaefer, Cosmet. *Toiletries*, 126 (2011) 462.

[12] European Commission. Scientific Committee on Consumer Safety (SCCS). Public Consultation on fragrance allergens in cosmetic products. Available at: http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/sccs_consultation_04_en.htm (Accessed March 2015).

[13] European Commission. Scientific Commitee on Consumer Safety (SCCS). Opinion on fragrance allergens in cosmetic products. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_073.pdf (Accessed March 2015).

[14] A. Schnuch, W. Uter, H. Dickel, C. Szliska, S. Schliemann, R. Eben, F. Ruëff, *Contact Dermatitis*, 61 (2009) 152.

[15] S.W.P. Wijnhoven, J. Ezendam, A.G. Schuur, H. Van Loveren, J.G.M. Van Engelen, RIVM report 320025001 (2008).

[16] D.A. Buckley, *Brit. J. Dermatol.*, 157 (2007) 295.

[17] S.C. Rastogi, Report Danish Ministry of the Environmental, Environmental Protection Agency (EPA), 2002.

[18] M.V. Heisterberg, T. Menné, J.D. Johansen, *Contact Dermatitis*, 70 (2014) 81.

[19] European Commission. Directive 2009/48/CE about toys safety.

[20] S.L. Simonich, W.M. Begley, G. Debaere, W.S. Eckhoff, *Environ. Sci. Technol*., 34 (2000) 959.

[21] D.R. Bickers, P. Calow, H.A. Greim, J.M. Hanifin, A.E. Rogers, J.H. Saurat, I. G. Sipes, R.L. Smith, H. Tagami, *Regul. Toxicol. Pharm*., 37 (2003) 218.

[22] Q. Hou, X. Sheng, J.H. Wang, Y.J. Liu, C.B. Liu, *BBA-Proteins. Proteom*., 1824 (2012) 263.

[23] J. Cheng, K.A. Zug, *Dermatitis*, 25 (2014) 232.

[24] J.C. da Rosa, D. Malajian, A. Shemer, M. Rozenblit, N. Dhingra, T. Czarnowicki, S. Khattri, B. Ungar, R. Finney, H. Xu, X. Zheng, Y.D. Estrada, X. Peng, M. Suarez-Fariñas, J.G. Krueger, E. Guttman-Yassky, *J. Allergy Clin. Immun*., 135 (2015) 712.

[25] European Commission. Scientific Commitee on Consumer Safety (SCCS). Opinion on hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC). Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_074.pdf (Accessed March 2015).

[26] D. Salvito, Environ. *Health. Persp*., 113 (2005) A802.

[27] C. Sommer, in Series Anthropogenic Compounds, ed. Springer, Berlin, 2004.

[28] Swedish Society for Nature Conservation. Fragrances 2000. Available at: http://www.leffingwell.com/rap-bmv-fragrances2000.pdf (Accessed March 2015).

[29] TNO Environment and Geosciences. TNO-Report. Phthalates and artificial musks in perfumes. Available at: https://secured-static.greenpeace.org/international/Global/international/planet-2/report/2005/2/phthalates-and-artificial-musk.pdf (Accessed March 2015).

[30] V. Homem, J.A. Silva, N. Ratola, L. Santos, A. Alves, *J. Chem. Technol. Biot*., (2015). DOI: 10.1002/jctb.4628.

[31] I. Weinberg, A. Dreyer, R. Ebinghaus, *Environ. Pollut*., 159 (2011) 125.

[32] M. Clara, O. Gans, G. Windhofer, U. Krenn, W. Hartl, K. Braun, S. Scharf, C. Scheffknecht, *Chemosphere*, 82 (2011) 1116.

[33] C. Garcia-Jares, J. Regueiro, R. Barro, T. Dagnac, M. Llompart, *J. Chromatogr. A.*, 1216 (2009) 567.

[34] A. Walters, D. Santillo, P. Johnston. Technical Report, GRL-TN-07-2005. Greenpeace Laboratories (2005).

[35] L. Duedahl-Olesen, T. Cederberg, K.H. Pedersen, A. Højgård, *Chemosphere*, 61 (2005) 422.

[36] J. Tomar, V.K. Jain, K. Aggarwal, S. Dayal, S. Gupta, *J. Dermatol*., 32 (2005) 951.

[37] R.H. Schreurs, E. Sonneveld, J.H. Jansen, W. Seinen, B. van der Burg, *Toxicol. Sci*., 83 (2005) 264.

[38] T. Luckenbach, D. Epel, *Environ. Health Persp*., (2005) 17.

[39] K.M. Taylor, M. Weisskopf, J. Shine, *Environ. Health*, 13 (2014) 14.

[40] X. Zhang, Y. Jing, L. Ma, J. Zhou, X. Fang, X. Zhang, Y. Yu, Int. *J. Hyg. Environ. Health*, 218 (2015) 99.

[41] A. Covaci, T. Geens, L. Roosens, N. Ali, N. Van den Eede, A.C. Ionas, G. Malarvannan, A.C. Dirtu, D. Barceló (Ed.) in Emerging Organic Contaminants and Human Health, Springer, Berlin (2012), 243.

[42] G. Wypych, in Handbook of plasticizers, ed. ChemTec Publishing, Canada (2004).

[43] European Council for plasticizers and intermediates. Committee to the Science of Safety. Disponible en: http://www.ecpi.org/about (consultado en marzo, 2015).

[44] E. Fasano, F. Bono-Blay, T. Cirillo, P. Montuori, S. Lacorte, *Food Control*, 27 (2012) 132.

[45] M.I. González-Castro, M.F. Olea-Serrano, A.M. Rivas-Velasco, E. Medina-Rivero, L.G. Ordoñez-Acevedo, A. De León-Rodríguez, *B. Environ. Contam. Tox*., 86 (2011) 627.

[46] J. Vikelsøe, M. Thomsen, L. Carlsen, *Sci. Total Environ*., 296 (2002) 105.

[47] R. Mankidy, S. Wiseman, H. Ma, J.P. Giesy, *Toxicol. Lett*., 217 (2013) 50.

[48] R. Bouchiat, E. Veignie, D. Grizard, C. Soebert, M. Vigier, C. Rafin, *Des. Water T*., (2015) 1.

[49] V. Matamoros, L.X. Nguyen, C.A. Arias, S. Nielsen, M.M. Laugen, H. Brix, *Water. Res*., 46 (2012) 3889.

[50] B. Prasad, S. Suresh, *Int. Biodeter. Biodegr*., 97 (2015) 179.

[51] A.S. Stasinakis, *Bioresource. Technol*., 121 (2012) 432.

[52] W. Jianlong, C. Lujun, S. Hanchang, Q. Yi, *Chemosphere*, 41 (2000) 1245.

[53] P. Ventrice, D. Ventrice, E. Russo, G. De Sarro, *Environ. Toxicol. Phar*., 36 (2013) 88.

[54] T. Schettler, *Int. J. Androl*., 29 (2006) 134.

[55] R.A. Rudel, D.E. Camann, J.D. Spengler, L.R. Korn, J.G. Brody, *Environ. Sci. Technol*., 37 (2003) 4543.

[56] J.A. Tickner, T. Schettler, T. Guidotti, M. McCally, M. Rossi, *Am. J. Ind. Med*., 39 (2001) 100.

[57] T. Cirillo, E. Fasano, F. Esposito, P. Montuori, R. Amodio Cocchieri, *Food. Chem. Toxicol*., 51 (2013) 434.

[58] Å. Bergman, J.J. Heindel, S. Jobling, K.A. Kidd, R.T. Zoeller, State of the science of endocrine disrupting chemicals 2012, World Health Organization (2013).

[59] J.R. Furr, C.S. Lambright, V.S. Wilson, P.M. Foster, L.E. Gray, *Toxicol. Sci*., 140 (2014) 403.

[60] S. Jones, A. Boisvert, T.B. Duong, S. Francois, P. Thrane, M. Culty, *Biol. Reprod*., 114 (2014) 1.

[61] M. De Falco, M. Forte, V. Laforgia, *Front. Environ. Sci*., 3 (2015) 1.

[62] B.C. Blount, M.J. Silva, S.P. Caudill, L.L. Needham, J.L. Pirkle, E.J. Sampson, G.W. Lucier, R.J. Jackson, J.W. Brock, *Environ. Health. Persp*., 108 (2000) 979.

[63] H.M. Koch, B. Rossbach, H. Drexler, J. Angerer, *Environ. Res*., 93 (2003) 177.

[64] R. Hauser, J.D. Meeker, N.P. Singh, M.J. Silva, L. Ryan, S. Duty, A.M. Calafat, *Hum. Reprod*., 22 (2007) 688.

[65] M.J. Silva, J.A. Reidy, A.R. Herbert, J.L. Preau, L.L. Needham, A.M. Calafat, *B. Environ. Contam. Tox*., 72 (2004) 1226.

[66] P.-C. Huang, P.-L. Kuo, Y.-Y. Chou, S.-J. Lin, C.-C. Lee, *Environ. Int*., 35 (2009) 14.

[67] J.D. Meeker, *Arch. Pediat. Adol. Med*., 166 (2012) 952.

[68] T.T. Schug, A.M. Blawas, K. Gray, J.J. Heindel, C.P. Lawler, *Endocrinology* (2015). doi:10.1210/en.2014-1734.

[69] M.G. Soni, I.G. Carabin, G.A. Burdock, *Food. Chem. Toxicol*., 43 (2005) 985.

[70] I. González-Mariño, J.B. Quintana, I. Rodríguez, S. Schrader, M. Moeder, *Anal. Chim. Acta*., 684 (2011) 59.

[71] P. Canosa, I. Rodríguez, E. Rubí, M.H. Bollaín, R. Cela, *J. Chromatogr. A*., 1124 (2006) 3.

[72] B. Albero, R.A. Pérez, C. Sánchez-Brunete, J.L. Tadeo, *J. Hazard. Mater*., 239 (2012) 48.

[73] C. Liao, S. Lee, H.-B. Moon, N. Yamashita, K. Kannan, *Environ. Sci. Technol*., 47 (2013) 10895.

[74] H. Yamamoto, I. Tamura, Y. Hirata, J. Kato, K. Kagota, S. Katsuki, A. Yamamoto, Y. Kagami, N. Tatarazako, *Sci. Total Environ*., 410 (2011) 102.

[75] M. Pedrouzo, F. Borrull, R.M. Marcé, E. Pocurull, *J. Chromatogr. A*., 1216 (2009) 6994.

[76] J. Cha, A.M. Cupples, *Water. Res*., 43 (2009) 2522.

[77] A.M.C. Ferreira, M. Möder, M.E.F. Laespada, *J. Chromatogr. A*., 1218 (2011) 3837.

[78] P. Canosa, I. Rodriguez, E. Rubí, R. Cela, *Anal. Chem*., 79 (2007) 1675.

[79] J. Regueiro, M. Llompart, C. Garcia-Jares, R. Cela, *J. Chromatogr. A*., 1174 (2007) 112.

[80] P.D. Darbre, P.W. Harvey, *J. Appl. Toxicol*., 28 (2008) 561.

[81] M.A. Calafat, X. Ye, L.Y. Wong, A.M. Bishop, L.L. Needham, Environ. *Health. Persp*., 118 (2010) 679.

[82] N.R. Janjua, H. Frederiksen, N.E. Skakkebaek, H.C. Wulf, A.M. Andersson, *Int. J. Androl*., 31 (2008) 118.

[83] H. Frederiksen, C. Taxvig, U. Hass, A.M. Vinggaard, C. Nellemann, *Toxicol. Sci*., 106 (2008) 376.

[84] C. Philippat, M.S. Wolff, A.M. Calafat, X. Ye, R. Bausell, M. Meadows, J. Stone, R. Slama, S.M. Engel, *Environ. Health Persp*., 121 (2013) 1225.

[85] E.P. Hines, P. Mendola, X. Ye, A.M. Calafat, S.E. Fenton, *Reprod. Toxicol*., 27 (2014) 239.

[86] P.D. Darbre, A. Aljarrah, W.R. Miller, N.G. Coldham, M.J. Sauer, G.S. Pope, *J. Appl. Toxicol*., 24 (2004) 5.

[87] P.W. Harvey, *J. Appl. Toxicol*., 24 (2004) 1.

[88] P.D. Darbre, *Best. Pract. Res. Cl. En*., 20 (2006) 121.

[89] E.J. Routledge, J. Parker, J. Odum, J. Ashby, J.P. Sumpter, *Toxicol. Appl. Pharm*., 153 (1998) 12.

[90] C. Lemini, R. Jaimez, M.E. Ávila, Y. Franco, F. Larrea, A.E. Lemus, *Toxicol. Ind. Health*., 19 (2003) 69.

[91] G.J. Nohynek, C.J. Borgert, D. Dietrich, K.K. Rozman, *Toxicol. Lett*., 223 (2013) 295.

[92] R.J. Witorsch, J.A. Thomas, *Crit. Rev. Toxicol*., 40 (2010) 1.

[93] J. Boberg, C. Taxvig, S. Christiansen, U. Hass, *Reprod. Toxicol*., 30 (2010) 301.

[94] A.L. Cashman, E.M. Warshaw, *Dermatitis* 16 (2005) 57.

[95] J. Chen, K.C. Ahn, N.A. Gee, S.J. Gee, B.D. Hammock, B.L. Lasley, *Toxicol. Appl. Pharm*., 221 (2007) 278.

[96] R.S. Tavares, F.C. Martins, P.J. Oliveira, J. Ramalho-Santos, F.P. Peixoto, *Reprod. Toxicol*., 27 (2009) 1.

[97] W. Chu, T.K. Lau, *J. Hazard. Mater*., 144 (2007) 249.

[98] A. Pop, C. Berce, P. Bolfa, A. Nagy, C. Catoi, I.B. Dumitrescu, F. Loghin, *Farmacia* 61 (2013) 202.

[99] A. Pop, B. Kiss, F. Loghin, *Clujul Medical* 86 (2014) 16.

[100] D.E. Latch, J.L. Packer, W.A. Arnold, K. McNeill, *J. Photoch. Photobio. A*., 158 (2003) 63.

[101] M. Lores, M. Llompart, L. Sanchez-Prado, C. Garcia-Jares, R. Cela, *Anal. Bioanal. Chem*., 381 (2005) 1294.

[102] Commission Regulation (EU) No 358/2014 of 9 April 2014 amending Annexes II and IV to Regulation (EC) 1223/2009 of the European Parliament and of the Council on cosmetic products, Off. J. Eur. Union L107/5. Available at: http://eur-lex.europa.eu/legal-content/ES/TXT/?uri=CELEX:32014R0358 (Accessed March 2015). Commission Regulation (EU) No 1004/2014 of 18 September 2014 amending Annex V to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products, Off. J. Eur. Union L282/5. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX: 32014R1004 (Accessed March 2015).

[103] Agence Nationale de Sécurité du Medicament et des Produits de Santé. Available at: http://ansm.sante.fr/S-informer/Points-d-information-Points-d-information/L-ANSM-recommande-de-restreindre-la-concentration-de-phenoxyethanol-dans-les-produits-cosmetiques-destines-aux-enfants-de-moins-de-3-ans-Point-d-information (Accessed February 2015).

[104] World Health Organization (WHO). Pesticides. Available at: http://www.who.int/topics/pesticides/es (Accessed March 2015).

[105] M.T. McGrath, *Am. Phytopathol. Soc. Retrieved*. 11 (2004) 1.

[106] T. Miguéns, M.C. Leirós, F. Gil-Sotres, C. Trasar-Cepeda, *Sci. Total. Environ*., 378 (2007) 218.

[107] A.L. Collins, D.F. McGonigle, *Environ. Sci. Policy*, 11 (2008) 97.

[108] H. Cotler, A. Garrido, V. Bunge, M.L. Cuevas, in: Las cuencas hidrográficas de México: diagnóstico y priorización, ed. Pluralia Ediciones e Impresiones, Mexico, 2010.

[109] Résidus de Pesticides dans les vins. In. Que choisir? Union Française de Consommateurs, October 2013.

[110] European Commission. EU Pesticides Database. Available at: http://ec.europa.eu/sanco_pesticides/public/?event=pesticide.residue.selection&language=EN (Accessed March 2015).

[111] Regulation No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels in or food and feed of plants and animal origin and amending Council Directive 91/414/EEC. Off. J. Eur. Union L70/1. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32005R0396&from=ES (Accessed March 2015).

[112] P. Cabras, A. Angioni, V.L. Garau, M. Melis, F.M. Pirisi, E.V. Minelli, F. Cabitza, M. Cubeddu, *J. Agr. Food Chem*., 45 (1997) 2708.

[113] S. Navarro, A. Barba, J. Oliva, G. Navarro, F. Pardo, *J. Agr. Food Chem*., 47 (1999) 264.

[114] S. Navarro, A. Barba, G. Navarro, N. Vela, J. Oliva, *J. Chromatogr. A*., 882 (2000) 221.

[115] J. Oliva, A.M. Marínez-Gil, C. Lorenzo, M.A. Cámara, M.R. Salinas, A. Barba, T. Garde-Cerdán, *Food. Chem*., 170 (2015) 401.

[116] R. Noguerol-Pato, T. Sieiro-Sampredro, C. González-Barreiro, B. Cancho-Grande, J. Simal-Gándara, *Molecules*, 19 (2014) 12173.

[117] M.L. Xu, J.B. Liu, J. Lu, *Appl. Spectrosc. Rev*., 49 (2014) 97.

[118] A.K. Haritash, C.P. Kaushik, *Asian J. Water. Environ. Pollut*., 8 (2011) 73.

[119] L.Y. Liu, P. Kukučka, M. Venier, A. Salamova, J. Klánová, R.A. Hites, *Environ. Int*., 64 (2014) 48.

[120] A. Bourgeault, C. Gourlay-Francé, *Sci. Total Environ*., 454 (2013) 328.

[121] Y. Hu, G. Li, M. Yan, C. Ping, J. Ren, *Sci. Total. Environ*., 473 (2014) 459.

[122] R.H. Peng, A.S. Xiong, Y. Xue, X.Y. Fu, F. Gao, W. Zhao, Y.S. Tian, Q.H. Yao, *FEMS. Microbiol. Rev*., 32 (2008) 927.

[123] A.K. Haritash, C.P. Kaushik, *J. Hazard. Mater*., 169 (2009) 1.

[124] S.Y. Yuan, L.C. Shiung, B.V. Chang, *B. Environ. Contam. Toxicol*., 69 (2002) 66.

[125] C.E. Boström, P. Gerde, A. Hanberg, B. Jernström, C. Johansson, T. Kyrklund, A. Rannug, M. Törnqvist, K. Victorin, R. Westerholm, *Environ. Health Persp*., 110 (2002) 451.

[126] C.L. Lemieux, A.S. Long, I.B. Lambert, S. Lundstedt, M. Tysklind, P.A. White, *Environ. Sci. Technol*., 49 (2015) 1797.

[127] K.H. Jung, B. Yan, S.N. Chillrud, F.P. Perera, R. Whyatt, D. Camann, P.L. Kinney, R.L. Miller, *Int. J. Environ. Res*., 7 (2010) 1889.

[128] A. Tarantini, A. Maître, E. Lefèbvre, M. Marques, A. Rajhi, T. Douki, *Toxicology*, 279 (2011) 36.

[129] Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foddstuffs, Off. J. Eur. Union L215/4. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=uriserv:OJ.L_.2011.215.01.0004.01.ENG (Accessed March 2015).

[130] Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending 2000/60/EC of the European Parliament and of the Council, Off. J. Eur. Union, L348/84. Available at: http://eur-lex.europa.eu/legal-content/ES/ALL/;ELX_SESSIONID=C5J1J5dp2NDyTnkTM2BnF16ZlvzmCHkxTB3lLP2Q41RLvLhCG5WQ!1141425537?uri=CELEX:32008L0105 (Accessed March 2015).

[131] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human comsumption, Off. J. Eur. Union, L330/32. Available at: https://www.fsai.ie/uploadedFiles/Legislation/Food_Legisation_Links/Water/EU_Directive_98_83_EC.pdf (Accessed March 2015).

[132] Real Decreto 1619/2005, de 30 de diciembre sobre la gestión de neumáticos fuera de uso, Boletín Oficial del Estado (BOE) del 3 de enero de 2006, 352. Available at: http://www.boe.es/boe/dias/2006/01/03/pdfs/A00352-00357.pdf (Accessed February 2015).

[133] M. Llompart, L. Sanchez-Prado, J.P. Lamas, C. Garcia-Jares, E. Roca, T. Dagnac, *Chemosphere*, 90 (2013) 423.

[134] M. Selbes, O. Yilmaz, A.A. Khan, T. Karanfil, *Chemosphere*, (2015). doi: 10.1016/j.chemosphere.2015.01.042.

[135] T. Haugland, R.T. Ottesen, T. Volden, *Environ. Pollut*., 153 (2008) 266.

[136] R.T. Ottesen, J. Alexander, M. Langedal, T. Haugland, E. Høygaard, *Environ. Geochem. Health*, 30 (2008) 623.

[137] B.J. Mahler, P.C.V. Metre, J.L. Crane, A.W. Watts, M. Scoggins, E.S. Williams, *Environ. Sci. Technol*., 46 (2012) 3039.

[138] J.L. Crane, *Arch. Environ. Cont. Tox*., 66 (2014) 176.

[139] E. García-Flores, F.T. Wakida, J.H. Espinoza-Gomez, *Int. J. Environ. Res*., 7 (2013) 387.

[140] S. Moret, S.C. Lanfranco, *J. Chromatogr. A*., 882 (2000) 245.

[141] F.J. Arrebola, A.G. Frenich, M.J. González-Rodríguez, P.P. Bolaños, J.L. Martínez-Vidal, J. *Mass. Spectrom*., 41 (2006) 822.

[142] V.H. Teixeira, S. Casal, M.B. Oliveira, *Food Chem*., 104 (2007) 106.

[143] P. Anastas, N. Eghbali, *Chem. Soc. Rev*., 39 (2010) 301.

[144] J. Regueiro, M. Llompart, C. Garcia-Jares, J.C. Garcia-Monteagudo, R. Cela, *J. Chromatogr. A*., 1190 (2008) 27.

[145] F.Priego-Capote, M.D. Luque de Castro in Analytical Applications of Ultrasounds, ed. Elsevier, The Netherlands, 2006.

[146] J.A. Perez-Serradilla, F. Priego-Capote, M.D. Luque de Castro, *Anal. Chem*., 79 (2007) 6767.

[147] J. Pawliszyn in Solid phase microextraction: theory and practice, ed. John Wiley & Sons, New York, 1997.

[148] T. Górecki, X. Yu, J. Pawliszyn, *Analyst*., 124 (1999) 643.

[149] N.P. Brunton, D.A. Cronin, F.J. Monahan, *Flavour. Frag. J*., 16 (2001) 294.

[150] J. Darrouzès, M. Bueno, C. Pécheyran, M. Holeman, M. Potin-Gautier, *J. Chromatogr. A*., 1072 (2005) 19.

[151] E. Psillakis, A. Mousouraki, E. Yiantzi, N. Kalogerakis, *J. Chromatogr. A*., 1244 (2012) 55.

[152] E. Psillakis, E. Yiantzi, L. Sanchez-Prado, N. Kalogerakis, *Anal. Chim. Acta*., 742 (2012) 30.

[153] E. Psillakis, E. Yiantzi, N. Kalogerakis, *J. Chromatogr. A*., 1300 (2013) 119.

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III. RESULTADOS Y DISCUSIÓN

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1. Determinación de sustancias potencialmente tóxicas en productos de cuidado personal

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Los productos cosméticos y de cuidado personal son empleados por millones de personas a diario en todo el mundo y cada vez son más numerosos los estudios que sugieren que algunos de los compuestos químicos empleados en sus formulaciones pueden tener efectos negativos para la salud.

En el momento en el que se inició este trabajo de investigación existía ya una creciente preocupación en cuanto a la seguridad de los ingredientes que pueden formar parte de los productos cosméticos y de cuidado personal y muchas marcas comenzaban a ofrecer productos con las etiquetas "*fragrance free*", "*phthalate free*" o "*non-paraben product*"; en este último caso, el referido a los parabenos, muchos autores los empezaban a clasificar como posibles disruptores endocrinos y en algunos países europeos como Dinamarca se desaconsejaba su uso como ingredientes de cosméticos; asimismo, tras la entrada en vigor en julio de 2013 de la mayoría de las disposiciones del Reglamento EC No 1233/2009 que introdujo numerosas restricciones y prohibiciones y que obligó a los fabricantes a reformular sus productos, era casi "obligatorio" desarrollar nuevos métodos de análisis robustos y fiables que permitiesen la detección y cuantificación de todos estos compuestos legislados y que pudiesen ser aplicados a un amplio rango de productos, incluyendo los destinados a la población infantil, ya que los métodos existentes hasta el momento eran escasos y la mayoría solo se centraba en la determinación de unos pocos compuestos en matrices muy concretas.

Por lo tanto, el ***principal objetivo*** del ***Capítulo I*** de esta Tesis era ***desarrollar un método analítico*** que permitiese el ***estudio simultáneo*** de un ***gran número*** de sustancias de distintas familias de ***diversa naturaleza química***, y que pudiese ser ***aplicado a*** un ***amplio rango*** de productos ***cosméticos*** y de cuidado personal. El principal problema que presentan las matrices cosméticas a la hora de abordar su análisis es su amplia variedad, ya que pueden presentarse en formas líquidas, viscosas, en polvos, como aerosoles… de hecho, los pocos métodos oficiales basados en GC-MS que existen hasta el momento para el análisis de estos productos se aplican solamente a muestras preparadas para inyectar directamente, como perfumes, donde la preparación de la muestra se hace por simple dilución en el disolvente adecuado[1,2]. En este trabajo, para la extracción de los compuestos de interés de las distintas formas cosméticas, se empleó la técnica de dispersión de matriz en fase sólida (MSPD), que ya había sido utilizada con éxito por el grupo de investigación en el que se ha desarrollado esta Tesis para analizar conservantes y fragancias alergénicas en cosméticos[3,4]. Para la determinación se empleó GC-MS; debido a la gran variedad de compuestos presentes en las formulaciones cosméticas, pueden existir problemas de coelución debido a interferencias de otras sustancias con las que se pretenden determinar. Algunos autores proponen el uso de dos columnas capilares cromatográficas para evitar problemas de coelución[5,6]. En este caso, como alternativa, se empleó la técnica de MS tanto en modo simple (MS) como en tándem (MS/MS), lo que permite "aislar" los compuestos de interés de las interferencias.

Uno de los primeros problemas surgió al emplear esta técnica en el análisis de los plastificantes (ftalatos y adipatos), ya que estos compuestos se encuentran presentes en todo el material plástico del laboratorio, así como en el aire. Para evitar problemas de contaminación y falsos positivos se decidió sustituir todo el material plástico por vidrio, y mantenerlo escrupulosamente limpio y a alta temperatura (230ºC) hasta su uso. Por lo tanto, durante el desarrollo de esta Tesis se empleó por primera vez una variación de la MSPD convencional que

conlleva una miniaturización de la misma (μ-MSPD), permitiendo obtener un método acorde con los principios de la "Química Verde", con un gasto mínimo de muestra y disolventes orgánicos y que apenas genera residuos, acortando además el tiempo de preparación de muestra y reduciendo costes. De esta forma, mediante esta nueva técnica de extracción y posterior análisis mediante cromatografía de gases-espectrometría de masas (GC-MS), se desarrolló y validó un método para el análisis simultáneo de ftalatos, adipatos y musks.

Al aplicar la μ-MSPD al análisis de fragancias alergénicas surgió el problema de que los compuestos más volátiles (*pinene* y *limonene*) no se extraían cuantitativamente posiblemente debido a su pérdida durante la etapa de disrupción de la muestra que se realizaba en mortero; para evitar la pérdida de estos analitos surgió la idea de realizar la disrupción en el propio vial donde se pesaba la muestra, lo que permitía además acortar la etapa de extracción. De esta forma, las recuperaciones obtenidas para *pinene* y *limonene* fueron cuantitativas, por lo que la metodología basada en ***μ-MSPD/GC-MS*** se pudo ***aplicar con éxito al análisis simultáneo de unos 70 compuestos de muy diversas características en cosméticos y productos de cuidado personal muy variados.*** También se utilizó la espectrometría de masas en tándem para mejorar la selectividad y sensibilidad analítica, ya que las matrices cosméticas son complejas y pueden existir interferencias a la hora de determinar los analitos de interés. De esta forma, la técnica MS/MS fue empleada por primera vez durante esta Tesis Doctoral para el análisis simultáneo de fragancias y conservantes en productos de cuidado personal, permitiendo disminuir notablemente los LODs.

Otro de los objetivos de este Capítulo se centró en estudiar un producto de cuidado personal destinado exclusivamente a la población infantil, como son las toallitas infantiles y el papel higiénico húmedo destinado a niños menores de 3 años. Este colectivo es mucho más vulnerable que la población adulta debido a que su barrera epidérmica no está completamente desarrollada y a la inmadurez de su sistema inmune. Además, estos productos son aplicados (hasta 16 unidades por día) sobre la zona genital, que a menudo se encuentra irritada y presenta un pH mayor, por lo que la permeabilidad de la piel a ciertas sustancias químicas se puede ver incrementada. Resulta curioso que hasta el momento no existía ninguna metodología ***para determinar fragancias, conservantes o plastificantes en*** este tipo de productos, ya que muchas de estas sustancias presentan restricciones especiales cuando se emplean en la formulación de productos destinados a niños menores de 3 años. Tanto las ***toallitas infantiles*** como el papel higiénico húmedo consisten en un soporte sólido (normalmente celulosa) impregnado con una loción, por lo que se propuso la ***extracción con líquidos presurizados (PLE) seguida de GC-MS*** como una técnica cómoda, rápida y eficaz para el análisis de 65 compuestos.

Es importante destacar que la optimización de las condiciones experimentales para las dos técnicas de extracción empleadas en los trabajos que se engloban en este Capítulo (μ-MSPD y PLE) se ha llevado a cabo mediante diseños experimentales lo que asegura una mayor eficacia en el proceso de extracción y asimismo, todos los métodos desarrollados se han validado en términos de linealidad, repetibilidad, reproducibilidad, exactitud y precisión.

En resumen, empleando extracción con µ-MSPD y PLE seguida de GC-MS y GC-MS/MS se han llevado a cabo los siguientes estudios que serán presentados a continuación:

- Análisis de plastificantes y almizcles sintéticos en cosméticos y productos de cuidado personal mediante dispersión de matriz en fase sólida seguida de cromatografía de gases-espectrometría de masas (*J. Chromatogr. A*., 1293 (2013) 10-19).

- Desarrollo de un método multianalito basado en micro-dispersión de matriz en fase sólida para el análisis de fragancias alergénicas y conservantes en productos de cuidado personal (*J. Chromatogr. A*., 1344 (2014) 1-14).

- Dispersión de matriz en fase sólida en vial para el análisis de fragancias alergénicas, conservantes, plastificantes y musks en cosméticos (*Cosmetics*, 1 (2014) 171-201).

- Extracción con líquidos presurizados-cromatografía de gases-espectrometría de masas para el análisis de fragancias alergénicas, musks, ftalatos y conservantes en toallitas infantiles (*J. Chromatogr.A*., 1384 (2015) 9-21).

BIBLIOGRAFÍA

[1] UNE-EN 16271:2013. Métodos para análisis de alérgenos. Cuantificación de fragancias sospechosas de ser alérgicas en productos de consumo. Parte 1: Análisis por GC de la muestra preparada para inyectar.

[2] UNE-EN 16521:2014. GC/MS para la identificación y ensayo de 12 ftalatos en muestras cosméticas listas para inyección analítica.

[3] L. Sanchez-Prado, J.P. Lamas, G. Alvarez-Rivera, M. Lores, C. Garcia-Jares, M. Llompart. J. Chromatogr. A., 1218 (2011) 5055.

[4] L. Sanchez-Prado, G. Alvarez-Rivera, J.P. Lamas, M. Lores, C. Garcia-Jares, M. Llompart, Anal. Bioanal. Chem 401 (2011) 3293.

[5] International Fragrance Association (IFRA) Analytical Procedure. Part 1, J. Agric. Food. Chem., 51 (2003) 6398.

[6] A. Chaintreau, E. Cicchetti, N. David, A. Earls, P. Gimeno, B. Grimaud, D. Joulain, N. Kupfermann, G. Kuropka, F. Saltron, C. Schippa, J. Chromatogr. A., 1218 (2011) 7869.

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1.1. ANALYSIS OF PLASTICIZERS AND SYNTHETIC MUSKS IN COSMETIC AND PERSONAL CARE PRODUCTS BY MATRIX SOLID-PHASE DISPERSION GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Journal of Chromatography A (Ed. Elsevier, Netherlands)
ISSN: 0021-9673

***J. Chromatogr. A., 1293 (2013) 10-19*. doi: 10.1016/j.chroma.2013.03.067**

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ANALYSIS OF PLASTICIZERS AND SYNTHETIC MUSKS IN COSMETIC AND PERSONAL CARE PRODUCTS BY MATRIX SOLID-PHASE DISPERSION GAS CHROMATOGRAPHY_MASS SPECTROMETRY

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ABSTRACT

Matrix solid-phase dispersion (MSPD) and gas chromatography-mass spectrometry were used for the rapid determination of 18 plasticizers (phthalates and adipates), 7 polycyclic musks and 5 nitromusks, which makes a total of 30 targets, in both rinse-off and leave-on cosmetic formulations. The MSPD method was miniaturized and customized to avoid or minimize risks of phthalate contamination and to reduce residues and costs. The amount of sample and extraction solvent employed were 0.1 g and 1 mL, respectively. The procedure was optimized by means of an experimental design and under the optimal conditions it showed satisfactory linearity, repeatability and intermediate precision. LOQs were, in general, in the low ng g^{-1}, and recoveries were quantitative for all the 18 plasticizers and the 12 fragrances.

Twenty-six cosmetic products such as creams, emulsions, lotions, gels for the skin, bath and shower preparations, deodorants, hair-setting, hair-cleansing and hair-conditioning products, shaving products, and sunbathing products, were analyzed. Twenty-five out of thirty targets were detected in the samples. The most frequently found compounds were galaxolide and tonalide reaching concentrations above 0.1 % (1000 μg g^{-1}), and diethyl phthalate (between 0.7 and 357 μg g^{-1}). The presence of banned substances (Regulation (EC) No 1223/2009) such as dibutyl phthalate, diisobutyl phthalate, dimethoxyethyl phthalate, benzylbutyl phthalate, diethylhexyl phthalate, diisopentyl phthalate and dipentyl phthalate, musk ambrette and musk tibetene was confirmed in sixteen of the twenty-six personal care products (62 %).

Keywords: Cosmetics; Musks; Phthalates; Plasticizers; Matrix solid-phase dispersion; Personal care products

1. INTRODUCTION

In Europe, the Regulation (EC) No 1223/2009 establishes the rules to be complied with by any cosmetic product available on the market, in order to ensure human health protection. It includes the list of the prohibited substances which must not be included in the cosmetic formulations, as well as the restrictions applied to other substances. In USA, the FDA is the organism responsible of dictaminating the rules to guarantee the health and welfare of consumers [1-3]. Cosmetic labelling requirements state that all cosmetics produced or distributed for retail sale to consumers for their personal care bear a list of ingredients, ordered by prevalence. But ingredients used in fragrances or chemical mixtures that are considered trade secrets are exempt from labelling requirements.

Phthalates (esters of phthalic acid) are a group of chemicals with a wide variety of industrial applications [4,5]. Regarding cosmetics, they are used as plasticizers in products such as nail polishes and hair sprays, and as solvents and perfume fixatives in many other products. This class of chemicals has been linked to hormone disruption, which can affect development and fertility [6]. Due to their potential risks for human health and environment, several of them have been included in the priority list of pollutants of different organizations. Although some phthalates such as dibuthyl phthalate (DBP) or diethylhexyl phthalate (DEHP) are being phased out of cosmetics, others such as diethyl phthalate (DEP) are still used without restrictions, in many products, including fragrances.

Fragrance is an obvious ingredient in perfumes, colognes, and deodorants, but it is used in nearly every type of personal care product. Fragrance recipes are considered trade secrets so manufacturers are not required to disclose fragrance chemicals in the list of ingredients. Typically, fragrances created for cosmetic and cleaning products are dominated by synthetic ingredients. The term "fragrance" or "parfum" on a cosmetic ingredients list usually represents a complex mixture of dozens of chemicals. Synthetic musks [7] exhibit a strong, warm, sensual and long-lasting odour, which makes them essential in modern perfumery and form the base note foundations of most perfume formulas. Nevertheless, synthetic musk fragrances have been described as a new group of bioaccumulative and persistent xenobiotics. Nitromusks dominated the market for many years but declined significantly in the 90 s [8] due to their bioaccumulative properties and health adverse reactions, which led to the prohibition of musk tibetene, musk moskene and musk ambrette. At the present, other two nitromusks, musk ketone and musk xylene are still permitted but with restrictions. There was a parallel increase in the use of polycyclic musks, a second group of synthetic musks which comprises several high volume use products, such as tonalide® (AHTN) and galaxolide® (HHCB). Although these compounds are still largely used in personal care products without restrictions [9], research indicate that the polycyclic musks are environmentally persistent, can accumulate in human bodies, and they are suspected hormone disruptors [1].

Some fragrance ingredients are not perfuming agents themselves but enhance the performance of perfuming agents. For example, diethyl phthalate (DEP) is widely used in cosmetic fragrances to make the scent linger. However, the European Commission on Endocrine Disruption has listed DEP as a Category 1 priority substance, based on evidence that it interferes with hormone

function [11]. Despite the potential health risks, phthalates are still choice ingredients in cosmetics because they are cheap and versatile.

In order to guarantee product safety according to regulations and to assess the health risk from the potential exposures, the development of analytical methods for the determination of phthalate esters and synthetic musks in cosmetic formulations is mandatory.

The increasing interest on the determination of the levels of both families of compounds in the environment, both considered emerging pollutants, is reflected by the number of studies on this matter in the last decade [12-14]. The determination of phthalates and musks in water has mainly been carried out by liquid-liquid extraction with organic solvents (LLE) [12-15]. Solid-phase extraction (SPE) also appears as a very suitable technique, since it requires fewer amounts of organic solvents, and permits the simultaneous extraction of multiple samples [12-14,16]. But applications of the conventional LLE and SPE are laborious and time consuming requiring large volumes of sample and organic solvents. Therefore, much attention is being paid to the development of miniaturized, more efficient, and environmentally friendly extraction techniques, which could greatly reduce residues and organic solvent consumption. Further, extraction, preconcentration and sample introduction can be performed in one step, as it is the case of solid-phase microextraction (SPME) [17-23], liquid-phase microextraction (LPME) [24,25], stir-bar-sorptive extraction (SBSE) [26,27] or ultrasound-assisted emulsification microextraction (USAEME) [28,29]. Apart from Soxhlet extraction, various advanced techniques as microwave assisted extraction (MAE) or pressurized fluid extraction (PFE) have been employed for the extraction of musk and phthalates from environmental solid samples [30-33]. Detection of both families of substances has mainly been carried out by GC-MS with conventional columns, although HPLC coupled to a mass spectrometry or UV detector has also been used. Recently the use of ionic liquids as GC stationary phase has been proposed for both plasticizers and musks [34].

Nevertheless, a look at the scientific literature evidences the lack of studies devoted to the development of methodology for the determination of musks in cosmetics, as well as the scarcity of studies about phthalates cosmetic analysis. In addition, in most of these studies the number of analytes considered is generally low. Sample preparation procedure usually consist on solvent extraction by mechanical shaking [6,35] or by sonication [36,37] followed by centrifugation or filtration. In some cases, a SPE clean-up or dilution step is included. The USAEME technique proposed by Regueiro et al. in 2008 [28] has recently been applied to the analysis of phthalate esters in cosmetics and environmental water samples [29]. Other studies regarding the analysis of phthalates, musks and other ingredients in cosmetic have been published but they only deal with the analysis of perfumes, and the samples were directly analyzed (or after dilution) by GC-MS [38,39]. Matrix solid phase dispersion (MSPD) has recently been proposed as a very suitable analytical tool for the extraction of preservatives and fragance allergens in cosmetic samples, providing efficient and low cost extractions, and meeting the requirements of the “green chemistry” [40-42].

The aim of this study is the development of a matrix solid phase dispersion (MSPD) method for the simultaneous determination of musks and phthalates in cosmetics. Both families of compounds are subjected to restrictions according international cosmetic regulations and,

therefore, the development of reliable analytical methodology is essential in cosmetic quality control. In the present study, we have also the intention of miniaturizing the technique, minimizing the consumption of sample, reagents and solvents, and avoiding the use of any special device and plastic material.

Table 1. Target compounds, purity, suppliers, CAS, retention time, selected MS ions, and EU cosmetic regulation [2].

Key	Compound	Purity (%)	CAS	Retention time (min)	Quantification and Identification ions	EU regulation
Plasticizers						
DMA	Dimethyl adipate	99[b]	627-93-0	6.91	101.0 (72), 111.0 (77), 114.0(100)	no restricted
DEA	Diethyl adipate	99[b]	141-28-6	8.30	111.0 (100), 128.0 (63), 157.1 (81)	no restricted
DMP	Dimethyl phthalate	98[c]	131-11-3	8.99	77.0 (13), 194.0 (6.6), 163.0 (100)	no restricted
DEP	Diethyl phthalate	98[c]	84-66-2	10.14	149.0 (100), 150.0 (12), 177.0 (24)	no restricted
DIBP	Diisobutyl phthalate	99[b]	84-69-5	12.15	57.0 (12), 149.0 (100), 223.1 (6.8)	prohibited
DBP	Dibutyl phthalate	99[b]	84-74-2	12.80	149.0 (100), 150.1 (9), 223.1 (4.9)	prohibited
DMEP	Dimethoxyethylphthalate	94[d]	117-82-8	13.03	59.1 (100), 104.0 (18), 149.0 (29)	prohibited
DIPP	Diisopentyl phthalate	99.5[d]	605-50-5	13.50	71.1 (23), 149.0 (100), 237.1 (10)	prohibited
DPP	Dipentyl phthalate	99.2[d]	131-18-0	14.00	71.1 (16), 149.0 (100), 237.1 (5.6)	prohibited
BBP	Benzylbutyl phthalate	98[e]	85-68-7	15.22	91.1 (53), 149.0 (100), 206.1 (24)	prohibited
DEHA	Di(2-ethylhexyl) adipate	98.5[b]	103-23-1	15.34	112.1 (26), 129.0 (100), 147.0 (21)	no restricted
DIHP	Diisoheptyl phthalate	99[e]	71888-89-6	15.74	149.0 (100), 223.0 (7), 265.1 (100)	no restricted
DEHP	Di(2-ethylhexyl)phthalate	99.5[c]	117-81-7	16.16	167.0 (30), 149.0 (100), 279.0 (10)	prohibited
DCHP	Dicyclohexyl phthalate	99[e]	84-61-7	16.19	55.0 (19), 149.0 (100), 167.0 (31)	no restricted
DPhP	Diphenyl phthalate	98[b]	84-62-8	16.36	77.0 (19), 153 (4), 225.0 (100)	no restricted
DOP	Di-n-octyl phthalate	≥98[c]	117-84-0	17.41	149.0 (100), 223.0 (22), 279.1 (6.2)	no restricted
DINP	Diisononyl phthalate	99[e]	28553-12-0	18.30	149.0 (100), 279.1 (7), 293.0 (17)	no restricted
DIDP	Disodecyl phthalate	99[c]	26761-40-0	19.01	71.0 (34), 149.0 (100), 307(20)	no restricted
Musks						
	Cashmeran	≥95[f]	33704-61-9	9.52	135.1 (43), 191.1 (100), 206.1 (57)	no restricted
	Celestolide	98[g]	13171-00-1	11.13	173.1 (22), 229.1 (100), 244.1 (44)	no restricted
	Phantolide	≥98[g]	15323-35-0	11.45	187.1 (11), 229.1 (100), 244.1 (24)	no restricted
MA	Musk Ambrette	99[d]	83-66-9	11.97	253.0 (100), 254.0 (13), 268.1 (35)	prohibited
	Traseolide	99[g]	68140-48-7	12.08	43.0 (41), 215.1 (100), 258.1 (14)	no restricted
	Galaxolide	50[f]	1222-05-5	12.15	213.0 (23), 243.1 (100), 258.1 (20)	no restricted
MX	Musk Xylene	100 ng mL^{-1} [h]	81-15-2	12.17	43.0 (62), 57.0 (16), 282.0 (100)	restricted[k]
	Tonalide	98[g]	1506-02-1	12.19	43.0 (48), 243.1 (100), 258.1 (26)	no restricted
MM	Musk Moskene	100 ng mL^{-1} [i]	116-66-5	12.35	263.1 (100), 264 (20), 278.1 (8.9)	prohibited
MT	Musk Tibetene	100 ng mL^{-1} [j]	145-39-1	12.70	43.0 (33), 251.1 (100), 266.1 (28)	prohibited
	Ambrettolide	≥97[f]	7779-50-2	12.78	67.0 (100), 81.0 (98), 96.1 (89)	no restricted
MK	Musk Ketone	100 ng mL^{-1} [h]	81-14-1	12.96	191.0 (24), 294.1 (26), 279.0 (100)	restricted[l]

[a]Numbers in brackets: relative ion abundances, %. [b]ChemService (West Chester, USA). [c]Fluka Chemie GmbH (Steinheim, Germany). [d]Dr.Ehrenstorfer(Ausburg, Germany). [e]Sigma-Aldrich Chemie GmbH (Steinheim, Germany). [f]Ventos (Barcelona, Spain). [g]LGC Standards GmbH (United Kingdom). [h]100 ng mL^{-1} in acetonitrile from Fluka Analytical (Germany). [i]100 ng mL^{-1} in acetonitrile from Riedel-de Haen (Germany). [j]100 ng mL^{-1} in cyclohexane from Dr. Ehrenstorfer (Ausburg, Germany). [k] Maximum concentration in ready for use preparation: 1.0% in fine fragrance, 0.4% in eau de toilette and 0.03% in other products. [l] Maximum concentration in ready for use preparation: 1.4% in fine fragrance, 0.56% in eau de toilette and 0.042% in other products. Grey cells: banned compounds (EC No 1223/2009).

2. EXPERIMENTAL

2.1. Chemicals, materials and samples

The studied compounds, their chemical names, CAS numbers, suppliers, purity, and the substance classification according EU Cosmetic Directive are summarized in **Table 1**. Deuterated bis(2-ethylhexyl)phthalate-3,4,5,6-d_4 (DEHP-d_4, 98atom % D), used as surrogate standard, was obtained from Fluka Chemie GmbH (Steinheim, Germany).

Acetone, ethyl acetate and n-hexane were provided by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Florisil (60-100 mesh) was purchased from Supelco Analytical (Bellefonte, PA, USA) and sodium sulphate anhydrous (99 %) from Panreac (Barcelona, Spain).

Individual stock solutions of each compound were prepared in acetone. Further dilutions and mixtures were prepared in acetone (sample fortification solutions), and ethyl acetate (calibration standards). All solutions were stored in amber glass vials at -20°C. All solvents and reagents were of analytical grade. The diluted solutions were prepared weekly.

All the glass, metallic and ceramic materials, the sorbents (Florisil and sodium sulphate anhydrous) and the glass wool for laboratory use (Sigma-Aldrich) were baked at 230°C for 12 h before use to eliminate possible phthalate contamination. All materials were allowed to cool down wrapped with aluminum foil [4,14,43]. Florisil and sodium sulphate anhydrous were allowed to cool down in a desiccator.

Cosmetics samples from national and international brands were purchased from local sources. They included leave-on and rinse-off products such as body milk, moisturizing creams, anti-aging creams, hand creams, sun milk, deodorant, shampoos and liquid soaps, hand soaps, among others. Samples were kept in their original containers at room temperature until their analysis.

2.2. MSPD procedure

0.1 grams of cosmetic were exactly weighted into a 10-mL glass vial and spiked with 10 µL of DEHP-d_4 surrogate solution (2.5 µg mL^{-1} in acetone). For the preparation of fortified samples, the cosmetic was spiked with 10 µL of the corresponding acetone solution of the target compounds to get the desired final concentration. Then, the sample was gently blended with 0.2 g of a drying agent (anhydrous Na_2SO_4) and 0.4 g of dispersing sorbent (Florisil) into a porcelain mortar using a porcelain pestle until a homogeneous mixture was obtained (ca. 5 min). The mixture was transferred into a glass Pasteur pipette (aprox. 150 mm), with a small amount of glass wool at the bottom, containing 0.1 g of Florisil (to obtain a further degree of fractionation and sample clean-up). Finally a small amount of glass wool was placed on top of the sample before compression with a small metallic spatula. Elution was made by gravity flow with ethyl acetate or hexane/acetone (1:1, v/v), collecting 1 mL of extract into a volumetric flask. The MSPD extracts, diluted when necessary in ethyl acetate, were directly analyzed by GC-MS. The experiments employing 0.5 g of sample were carried out in similar way but the device employed to hold the sample was a 15 mL polyethylene column with polyethylene frits. Likewise, the amounts of sorbents and solvents were proportionally incremented (5 times). The final optimized methodology comprised the MSPD extraction of 0.1 g of sample (0.2 g of anhydrous Na_2SO_4 and 0.4 g of Florisil) using Pasteur pipettes and glass wool plugs. The elution was made with 1 mL of ethyl acetate.

2.3. GC-MS analysis

The GC–MS analysis was performed using an Agilent 7890A (GC)-Agilent 5975C inert MSD with triple axis detector and an Agilent 7693 autosampler from Agilent Technologies (Palo Alto, CA, USA). The temperatures of the transfer line, the quadrupole and the ion source were set at 290, 150 and 230°C, respectively. The system was operated by Agilent MSD ChemStation E.02.00.493 software.

Separation was carried out on a cross-linked 5 %-phenyl/95 %-dimethylpolysiloxane SLB^{TM}-5ms capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness) obtained from Supelco Analytical (Bellefonte, PA, USA). Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 mL min^{-1}. The GC oven temperature was programmed from 80°C (held 2 min) to 290°C (held 10 min) at 15°C min^{-1}. Pulsed splitless mode was used for injection (30 psi, held 1 min). After 1 min the split valve was opened (75 mL min^{-1}) and the injector temperature was kept at 280°C. The injection volume was 1 µL. The mass spectra detector (MSD) operated in selected ion monitoring (SIM) mode, monitoring three ions per compound (**Table 1**). The electron multiplier was set at a nominal value of 1376 V.

2.4. Statistical analysis

Basic and descriptive statistics, as well as experimental design analysis were performed using Statgraphics-Plus v5.1 (Manugistics, Rockville, MD, USA) as software package. The experimental design was applied in the optimization of the extraction method, to analyze the simultaneous effect of the experimental parameters affecting MSPD.

3. RESULTS AND DISCUSSION

The chromatographic conditions were optimized to achieve an efficient separation of the 30 target compounds, 18 plasticizers and 12 musk fragrances. The MS detector was operated in the SIM mode selecting three ions per compound. The retention times and the quantifier and qualifier ions are shown in **Table 1**. **Figure 1** shows a chromatogram of a 100 ng mL^{-1} standard solution in ethyl acetate (DIHP, DINP, and DIDP 1000 ng mL^{-1}).

3.1. Method development

The application of MSPD for the extraction of different cosmetic additives has recently been proposed for the extraction of phenol preservatives [40] and allergen fragrances [41]. In the first case, a derivatization step was included. In both studies the optimized extraction experimental conditions were equivalent. The final conditions comprised the use of 0.5 g of sample mixed with 1 g of desiccant (anhydrous sodium sulphate), and 2 g of Florisil as dispersant agent. The MSPD column was then eluted with 5 mL of hexane/acetone. These developments have permitted the use of a single MSPD method for the determination of both families of compounds [44].In the present study we aimed to extend the application of MSPD for the extraction of two important families of cosmetic ingredients: the musk fragrances and the plasticizers, including main phthalates. Initially, we started applying the conditions optimized in previous studies with the further intention of developing a common multianalyte method suitable for several groups of cosmetic additives. We had other

additional main objectives in mind: to miniaturize the technique, to avoid the use of plastic, to generate the minimal amount of residues, and to reduce, even more, the sample preparation costs.

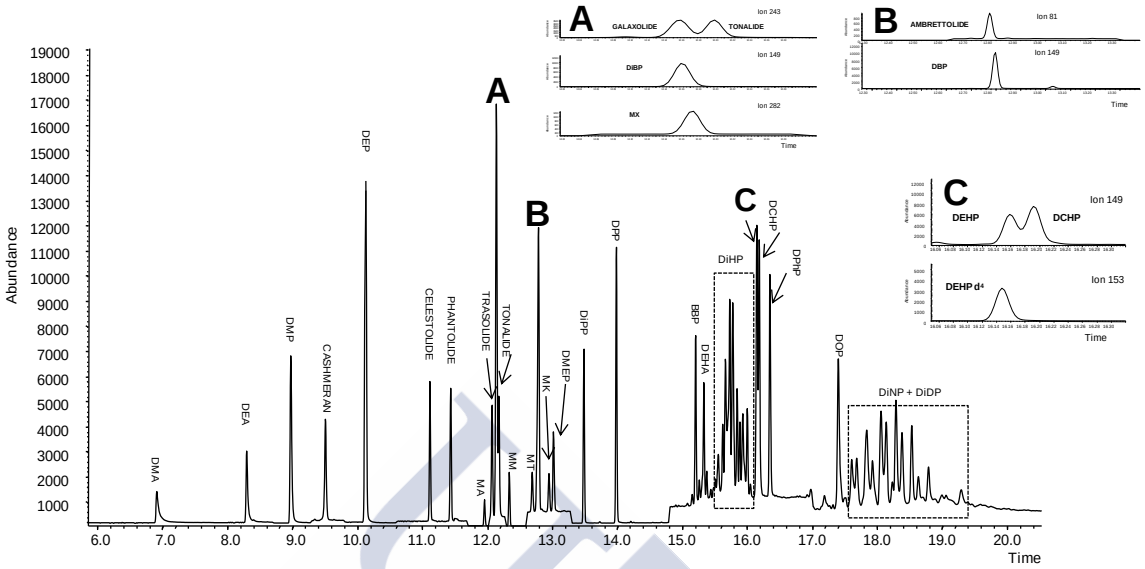

Figure 1. Chromatogram of a 100 ng mL^{-1} standard solution of all target analytes in ethyl acetate (DIHP, DINP and DIDP 1000 ng mL^{-1}).

3.1.1. MSPD optimization

A multivariate experimental strategy was carried out in order to select optimal extraction conditions. On the basis of our previous experience, some experimental parameters were kept fixed. Florisil was used as dispersant (4:1, w/w), and the volume of eluting solvent was 10 times (v/w) the sample size. Since drying of the sample is essential for an efficient extraction and chromatography, the samples were mixed with anhydrous sodium sulphate (2:1, w/w). Based on previous studies, two solvents (factor C) were investigated: hexane/acetone (1:1, v/v) and ethyl acetate. As we intended to develop a general method applicable to both categories of cosmetic samples, leave-on and rinse-off, a real sample of each type (factor A) was included in this study: a moisturizing baby lotion (leave-on) and a liquid bath soap (rinse-off) with negligible levels of the target analytes. The samples were fortified with all analytes (20 μg g^{-1}). The last factor included was the sample size (factor B). This factor was studied at two levels: 0.5 g and 0.1 g. The first level, 0.5 g, was the standard amount used in our previous research. In those cases, 15 mL plastic columns, with polypropylene frits at the bottom and top of the sample mixture, were employed. The low level of sample, 0.1 g, was included in the experimental design with the intention of miniaturizing the procedure. Glass Pasteur pipettes (aprox 150 mm, 1.5 mL), with a small amount of glass wool at the bottom and top, were used as sample column support devices. A factorial experimental design 2^3 was carried out. Two of the assays were performed in duplicate, gaining sufficient degrees of freedom to evaluate the statistical significance of main and second order factors (interactions). **Table 2** summarizes the factors and levels included in the experimental design. Data analysis was made with the statistical software package Statgraphics-Plus v5.1. The analysis of variance (ANOVA) for the thirty target compounds is shown in **Table 3**. The F-ratio measures the contribution of each factor or interaction on the variance

Table 2. Factors and levels considered in the experimental design.

Factor	Code	Low level (-)	High level (+)	Continuous
Sample	A	Rinse-off	Leave-on	No
Size (g)	B	0.1	0.5	Yes
Solvent	C	Hexane/Acetone	Ethyl acetate	No

Table 3. *F* ratios and *p* values obtained in the analysis of variance*

Plastizicers	A: Sample		B: Size		C: Solvent		AB		AC		BC	
	F ratio	*p* value	*F* ratio	*p* value	*F* ratio	*p* value	*F* ratio	*p* value	*F* ratio	*p* value	*F* ratio	*p* value
DMA	0.14	0.738	1.38	0.325	6.35	0.086	2.26	0.229	2.53	0.210	0.00	0.997
DEA	0.00	0.972	0.01	0.932	0.09	0.789	6.32	0.087	0.02	0.908	0.29	0.627
DMP	0.15	0.723	0.77	0.446	2.08	0.245	**16.24**	**0.028**	0.02	0.902	1.94	0.258
DEP	0.33	0.607	0.04	0.862	0.13	0.739	2.96	0.184	0.16	0.719	0.17	0.711
DIBP	0.40	0.574	1.32	0.334	0.02	0.904	8.65	0.061	0.34	0.599	0.40	0.573
DBP	4.64	0.120	0.04	0.861	1.89	0.263	2.92	0.186	0.13	0.738	0.02	0.899
DMEP	1.11	0.369	2.30	0.227	0.21	0.676	**12.46**	**0.039**	0.14	0.737	4.40	0.127
DIPP	0.81	0.435	0.06	0.819	0.13	0.744	2.77	0.195	0.44	0.555	0.37	0.587
DPP	0.52	0.523	1.78	0.274	2.99	0.182	8.91	0.058	0.38	0.580	2.06	0.246
BBP	5.58	0.099	0.21	0.675	5.42	0.102	9.18	0.056	3.37	0.164	0.64	0.482
DEHA	2.84	0.191	**16.66**	**0.027**	2.59	0.206	**42.03**	**0.008**	0.78	0.443	**24.47**	**0.016**
DIHP	0.03	0.871	0.70	0.463	0.33	0.605	0.99	0.394	0.01	0.923	0.19	0.691
DCHP	0.20	0.684	2.47	0.214	3.31	0.166	**17.42**	**0.025**	0.29	0.627	0.73	0.456
DEHP	3.43	0.161	0.70	0.463	0.37	0.586	7.79	0.068	0.89	0.414	0.67	0.473
DPhP	0.77	0.444	0.13	0.745	0.18	0.698	8.59	0.061	0.06	0.827	0.89	0.415
DOP	3.90	0.143	6.30	0.087	1.29	0.339	5.03	0.111	0.11	0.766	3.15	0.174
DINP	1.54	0.302	3.49	0.158	0.21	0.679	6.37	0.086	0.27	0.642	4.18	0.133
DIDP	10.80	0.081	0.83	0.459	3.06	0.222	1.48	0.348	0.14	0.741	3.93	0.186
Musks												
Cashmeran	0.05	0.835	0.44	0.556	0.93	0.407	6.55	0.083	0.22	0.674	0.73	0.456
Celestolide	0.30	0.621	0.00	0.964	0.50	0.530	7.13	0.076	0.00	0.967	0.53	0.519
Phantolide	0.09	0.780	0.01	0.921	0.11	0.767	4.47	0.125	0.12	0.748	0.35	0.596
MA	7.72	0.069	0.22	0.671	0.94	0.405	6.38	0.086	1.76	0.276	2.90	0.187
Traseolide	0.80	0.438	0.08	0.797	1.07	0.377	**11.29**	**0.044**	1.54	0.303	1.08	0.374
Galaxolide	0.62	0.487	1.71	0.282	0.08	0.800	7.74	0.069	0.43	0.557	0.41	0.566
Tonalide	0.00	0.996	1.83	0.269	0.01	0.923	8.24	0.064	0.63	0.487	0.57	0.504
MX	0.30	0.623	0.48	0.539	1.21	0.352	8.79	0.059	2.69	0.200	0.23	0.662
MM	2.04	0.248	0.24	0.656	2.01	0.251	8.31	0.063	0.91	0.411	0.97	0.396
Ambrettolide	0.40	0.574	0.33	0.608	0.59	0.498	0.76	0.447	1.44	0.317	0.17	0.705
MK	2.89	0.188	0.31	0.614	0.93	0.406	2.71	0.198	0.00	0.968	1.00	0.392

*Values in bold denote statistical significance. Grey cells: banned compounds (EC No 1223/2009).

of the response. The p-value tests the statistical significance of each factor and interaction. As can seen, the main factors sample type, sample size, and eluting solvent were no significant for both families of compounds, with p values higher than 0.05, with the only exception of sample size for DEHA. The second order factors AC and BC were also no significant (excluding BC for DEHA). The last second order factor AB was the predominant factor (see F values) although was only significant for five of the thirty studied compounds: four plasticizers (DMP, DMEP, DEHA and DCHP), and one musk (traseolide).

The design results can be displayed using several graphic tools as are the Pareto charts, the main effects diagrams and the interaction plots. Only those graphics showing significant factors are displayed. **Figure 2** includes the Pareto charts of the five compounds with statistically significant factors.

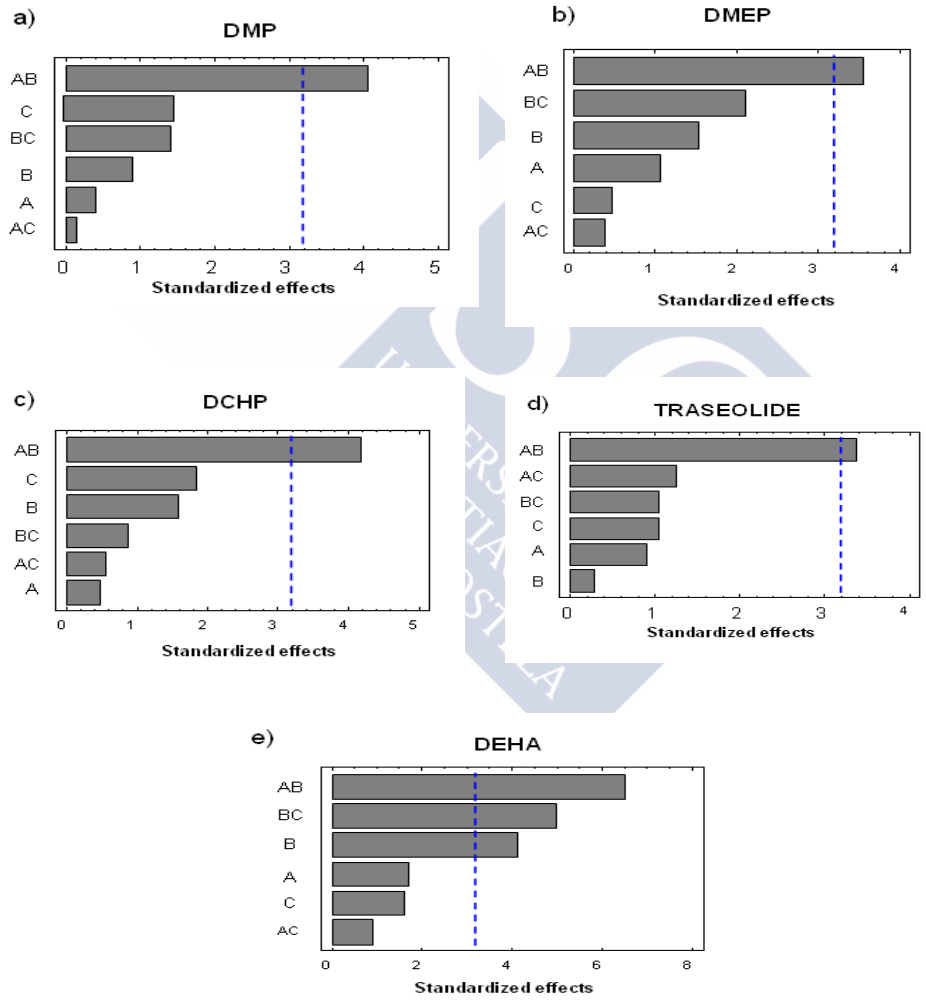

Figure 2. Pareto charts showing the significant factors (95%) for plasticizers and musks (factor codes: Table 2).

In these graphics the length of each bar is proportional to the absolute value of its associated standardized effect. The standardized effect is obtained by dividing the estimated effect of each factor or interaction by the standard error. Vertical line in the graphs represents the

statistically significant bound at the 95% confidence level. As previously mentioned, AB was the predominant factor for all compounds as well as the only significant factor for four of the five compounds. This interaction effect is illustrated in **Figure 3**. The two-factor plots display the least squares means at all combinations of two factors, which allows studying the effect of both factors simultaneously. The interaction graphics are very useful and help to visualize if there are interactions between the studied variables and determinate optimal conditions. As can be observed, the most favorable sample size was 0.5 g in the rinse-off experiments (+ code) whereas 0.1 g is most suitable for the leave-on sample (- code). For DEHA the factors size and the BC interaction were also significant (see Pareto chart in **Figure 2**). Regarding the sample size (**Figure 3f**) the use of 0.1 g appears as most favorable (higher response). Concerning BC interaction (**Figure 3e**) ethyl acetate (+ code) is the most suitable solvent for the extraction of 0.1 g of sample.

In view of the results, and although in most cases other conditions seem to be also suitable, the selected general conditions for the simultaneous extraction of the target musk fragrances and plasticizers both in leave-on and rinse-off cosmetics comprised the use of 0.1 g of sample and the elution with ethyl acetate. The selection of 0.1 g of sample implies significant advantages as it involves the use of only 0.4 g of Florisil as dispersant (instead of 2 g), 0.2 g of anhydrous sodium sulphate (instead of 1 g), and avoids the use of plastic materials (plastic columns and frits). As commented, the experiments are made using Pasteur pipettes and glass wool. It permits to reduce costs and minimize phthalate background levels because no plastics are used, and all the material can be baked at high temperatures before use (230°C, 12 h). Regarding the amount of solvent, it was drastically reduced from 5 mL to only 1 mL since the collection of a second fraction of 1 mL was unnecessary (the amount of analyte was less than 2 % in all cases).

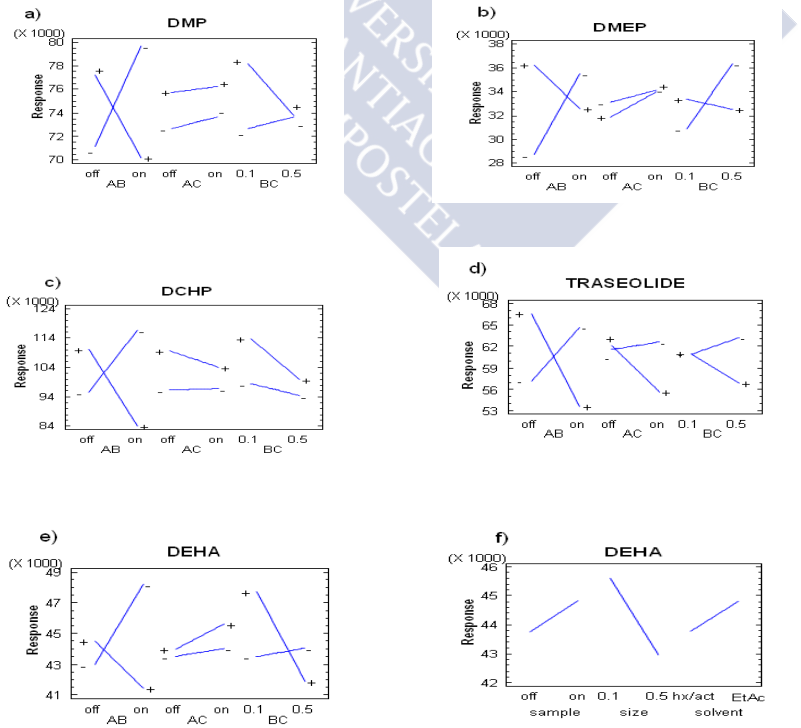

Figure 3. Interaction effects and main effects plots (see codes in Table 2).

3.2. Method performance

The GC-MS method performance parameters are summarized in **Table 4**. Regarding the instrumental linearity external standard calibration was carried out. Calibration standards in ethyl acetate were prepared covering a concentration range from 2 to 500 ng mL^{-1}, with the exception of DIHP, DINP and DIDP (50-5000 ng mL^{-1}). The method exhibited a direct proportional relationship between the concentration of each analyte and the chromatographic response (area counts). Correlation coefficients R≥0.997 for plasticizers and R≥0.993 for the synthetic musks were obtained. Method precision on standard solutions was studied within a day (n=3) and among days (n=5) at two concentration levels (20 and 200 ng mL^{-1}). For plasticizers, RSD values ranged from 0.17 to 9.6% (intra-day precision, average 2.7%), and between 2.6 and 15% (inter-day precision, average 7.7%). Precision for musk fragrances was also satisfactory with RSD values ranging from 0.10 to 3.1% for intra-day and 4.1 and 11% for inter-day studies (the averages for intra-day and inter-day precision were 1.0 and 7.9%, respectively). Instrumental detection limits (IDLs) were calculated as the concentration giving a signal-to-noise ratio of three (S/N = 3) in all cases since none of the target compounds were detected in the solvent chromatographic blanks. The obtained values are included in **Table 4** and they were below one ng mL^{-1}, excluding DIHP, DINP, and DIDP. It should be beard on mind that these three compounds are complex mixtures of isomers, and consequently, the chromatographic signal is made of many peaks, which explains the much higher detection limits.

Method quality parameters were evaluated using real cosmetic samples and are also shown in **Table 4**. In this way, recovery studies were carried out by applying the optimized method to the extraction of three real samples, including both types rinse-off and leave-on cosmetic products, spiked at 1 μg g^{-1} (4 μg g^{-1} for DINP and DIDP): a liquid soap (LS2), a body milk (BM), and a straightening hair cream (HC). The first two samples were also spiked at 10 μg g^{-1} (40 μg g^{-1} for DINP and DIDP). Previous analyses of the samples showed the presence of some of the target compounds, and these initial concentrations were taken into account to calculate the recoveries. As can be seen in **Table 4**, recoveries were higher than 87 % for all the target plasticizers and than 76 % for the musk fragances in all samples. Average recoveries were between 84-105 % in all cases. Precision was also evaluated in all samples and RSD values were generally lower than 8 % for all compounds in all samples, with an average value of 5.9% (see RSD values in **Table 4**).

Blank procedure analyses were carried out daily. Although all possible precautions were taken, the presence of some phthalates could not be avoided. The limits of detection (LODs) and quantification (LOQs) of the overall method were calculated as the compound concentration giving a signal-to-noise ratio of three (S/N=3) and ten (S/N=10), respectively, excluding those compounds detected in the whole method blanks, DEP, DIBP, DBP, and DEHP. LODs and LOQs for these compounds were estimated as the average amount of analyte giving a response that is the blank signal plus 3 or 10 times, respectively, the standard deviation (LOD = blank signal + 3SD; LOQ = blank signal + 10SD). As shown in **Table 4**, LOD values for the plasticizers ranged from 1.4 ng g^{-1} to 17 ng g^{-1}, with the exception of DIHP (50 ng g^{-1}), DINP (120 ng g^{-1}) and DIDP (300 ng g^{-1}). For musk compounds, LODs ranged from 1.9 to 12 ng g^{-1}, with the exception of ambrettolide (37 ng g^{-1}).

Table 4. Quality parameters of the method.

	Correlation coefficient (R)	IDL (ng mL^{-1})	Recoveries (RSD) (%)						LOD (% w/w x10^4)[c]	LOQ (% w/w x10^4)[c]
			LS2[a]	BM[a]	LS2[b]	BM[b]	HC[b]	Mean		
Plasticizers										
DMA	0.9994	0.90	85.7 (6.1)	88.0 (6.4)	103 (11)	110 (0.2)	87.7 (14)	100 (12.5)	0.0093	0.031
DEA	0.9992	0.58	115 (2.1)	90.0 (5.1)	109 (2.5)	105 (16)	104 (14)	104 (15.0)	0.0083	0.028
DMP	0.9996	0.21	93.3 (1.9)	76.7 (7.1)	96.5 (4.8)	88.9 (0.3)	88.9 (7.4)	88.9 (4.3)	0.0025	0.0082
DEP	0.9996	0.60	90.5 (2.1)	78.9 (8.5)	92.2 (4.4)	88.5 (2.0)	85.5 (8.5)	87.1 (5.1)	0.053	0.068
DIBP	0.9992	0.15	84.3 (1.3)	82.3 (7.0)	84.2 (5.7)	98.4 (6.5)	102 (6.9)	90.2 (5.5)	0.081	0.14
DBP	0.9990	0.15	86.5 (2.1)	93.7 (8.3)	87.5 (5.4)	95.0 (1.7)	111 (7.0)	94.7 (4.9)	0.080	0.18
DMEP	0.9991	0.65	103 (1.3)	106 (2.3)	112 (0.8)	80.6 (8.9)	111 (4.6)	102 (3.6)	0.0081	0.027
DIPP	0.9983	0.30	92.9 (3.4)	101 (9.0)	95.5 (7.3)	102 (5.1)	99.6 (7.6)	98.2 (6.5)	0.0069	0.023
DPP	0.9982	0.15	88.3 (4.5)	112 (6.8)	94.6 (9.0)	113 (1.5)	120 (5.4)	105 (5.4)	0.0016	0.0054
BBP	0.9976	0.45	90.7 (3.5)	102 (3.2)	106 (8.5)	100 (4.7)	119 (3.6)	103 (4.7)	0.0054	0.018
DEHA	0.9974	0.39	77.4 (8.2)	119 (6.3)	101 (12)	119 (15)	98.7 (14)	103 (14)	0.0056	0.019
DIHP	0.9989	3.0	79.6 (6.5)	99.5 (1.5)	104 (7.0)	100 (1.5)	103 (3.6)	97.2 (4.0)	0.050	0.17
DEHP	0.9976	0.65	78.8 (4.9)	108 (5.3)	110 (0.4)	106 (12)	103 (14)	101 (7.3)	0.082	0.15
DCHP	0.9990	0.60	79.2 (3.0)	90.2 (10)	88.0 (8.3)	92.2 (0.7)	120 (6.4)	93.9 (5.7)	0.0060	0.020
DPhP	0.9990	0.14	81.3 (2.7)	86.5 (11)	95.8 (4.7)	90.1 (5.1)	111 (5.9)	92.9 (5.9)	0.0014	0.0045
DOP	0.9966	0.30	82.1 (10)	104 (2.6)	97.2 (8.8)	89.2 (16)	112 (9.6)	96.9 (9.4)	0.0051	0.017
DINP	0.9972	12	82.0 (9.0)	108 (1.6)	99.2 (5.5)	105 (1.9)	102 (9.0)	99.2 (5.4)	0.12	0.40
DIDP	0.9979	25	79.4 (12)	108 (9.7)	101 (20)	112 (3)	n.c.	100 (11)	0.30	1.0
Musks										
Cashmeran	0.9996	0.60	103 (2.4)	83.7 (7.0)	102 (12)	95.0 (1.2)	77.9 (5.0)	92.3 (5.5)	0.0012	0.0041
Celestolide	0.9983	0.17	104 (3.9)	84.6 (8.2)	96.7 (4.9)	97.7 (4.0)	107 (9.0)	98.0 (6.0)	0.0020	0.0066
Phantolide	0.9983	0.16	93.7 (1.8)	83.8 (6.5)	89.9 (4.6)	98.7 (2.0)	119 (3.7)	97.0 (3.7)	0.0020	0.0067
MA	0.9965	0.39	95.2 (3.8)	80.2 (4.1)	91.5 (3.8)	117 (15)	118 (10)	100 (7.3)	0.0058	0.019
Traseolide	0.9970	0.60	97.1 (2.1)	86.9 (6.9)	95.6 (4.5)	97.9 (2.4)	115 (4.7)	98.5 (4.1)	0.0081	0.027
Galaxolide	0.9995	0.35	86.7 (2.9)	83.4 (8.5)	87.0 (4.6)	101 (1.4)	n.c.	89.7 (4.4)	0.0052	0.011
MX	0.9946	0.47	89.4 (4.6)	76.7 (4.3)	79.3 (3.5)	77.7 (8.7)	102 (11)	85.0 (6.4)	0.0049	0.016
Tonalide	0.9992	0.35	83.7 (2.7)	75.9 (9.2)	82.0 (5.5)	94.3 (0.6)	n.c.	83.9 (4.5)	0.0039	0.011
MM	0.9933	0.17	89.8 (5.8)	88.0 (5.3)	82.1 (3.1)	83.2 (13)	112 (6.8)	91.0 (6.8)	0.0019	0.0063
MT	0.9964	0.30	87.5 (6.2)	89.2 (6.5)	79.5 (4.4)	94.8 (9.7)	109 (8.0)	92.0 (6.9)	0.0035	0.011
Ambrettolide	0.9990	1.2	89.7 (0.5)	104 (3.9)	101 (15)	90.6 (0.8)	104 (4.6)	97.1 (2.5)	0.037	0.13
MK	0.9954	0.30	89.3 (4.6)	94.4 (5.5)	94.9 (1.4)	95.7 (6.1)	116 (8.0)	98.0 (5.1)	0.0030	0.010

IDL: Instrumental detection limits. LS: Liquid soap, BM: Body milk, HC: Hair conditioner. [a] 10 µg g^{-1} and DINP and DIDP 40 µg g^{-1}. [b] 1 µg g^{-1} and DINP and DIDP 4 µg g. [c]Equivalent to µg g^{-1}. Grey cells: banned compounds (EC No 1223/2009). n.c.: not calculated.

3.3. Application to real samples

The proposed method was applied to the analysis of 26 real cosmetic samples including 7 rinse-off (shampoos, liquid soaps, hair conditioners, and a body scrub) as well as 19 leave-on (body milks, moisturizing lotions, sun block, hands cream, anti aging cream, aftershave and deodorants among others) products, with the intention of demonstrating method adequacy for the wide variety of the most common cosmetic products. We have not included perfumes, colognes and eau the toilette, the cosmetics with, obviously, the highest concentrations of musks and DEP (due to its use as fragrance solvent), since these cosmetic formulations do not require any sample pretreatment other than dilution before GC-MS analysis [38]. Results are shown in **Tables 5 and 6** for rinse-off and leave-on cosmetics, respectively. For all the samples, the recoveries of DEHP-d_4 (surrogate standard) were satisfactory, with values generally higher than 80%, with an average value of 97 % for both rinse-off and leave-on samples.

3.3.1. Plasticizers

In the case of rinse-off cosmetics (**Table 5**), DEP was found in two samples (2.5 and 0.72 μg g^{-1}). DEHA was also found in two samples at low levels (< 0.2 μg g^{-1}). Three banned phthalates DIPP, DPP and BBP (Regulation EC No 1223/2009) were found in one sample, although at very low levels (closer or below LOQ).

Table 5. Analysis of rinse-off samples[a] (% w/w x10^4)[b]

	HC1	HC2	SH1	SH2	LS1	LS2	BS
Plasticizers							
DMA						<LOQ	
DEP		0.716		2.47			
DIPP	0.154						
DPP						<LOQ	
BBP						<LOQ	
DEHA	0.126					0.0448	
Musks							
Phantolide				6.54			
M. Ambrette							0.419
Traseolide		0.378					
Galaxolide	0.0496	0.0795	0.0374	0.134	0.0717	0.0358	0.0901
Tonalide		0.0483	0.0115	1760	0.0115	<LOQ	0.0329
$DEHPd_4$[c]	82.3	94.3	110	92.4	97.0	108	96.8

[a] HC: hair conditioner, SH: shampoo, LS: liquid soap. BS: body scrub. [b] Equivalent to μg g^{-1}. [c] Surrogate recovery (%). Blank cells: below LOD. Grey cells: banned compounds (EC No 1223/2009).

Regarding the leave-on samples (**Table 6**), the general plasticizers content was clearly higher. DEP was the most abundant compound (found in 53% of the samples), with values between 0.6 and 357 μg g^{-1}. This result confirms findings of several earlier studies [6,45] in which DEP was the most frequently used phthalate in cosmetic and personal care products (mainly used as a fragrance component). Also, 7 banned phthalates included in this study were found in some of the samples; two of them, DEHP and DBP were found in 7 of the 19 studied samples (37%). In addition, the levels

of DBP were quite high in three of the samples (from 10 to 141 μg g^{-1}) taking into account that this substance is of forbidden use in cosmetic products under the EU Cosmetics Directive. Also the levels of DEHP (25.8 μg g^{-1}) and DMEP (12.6 μg g^{-1}) found in two samples, both also prohibited compounds, were quite high.

Table 6. Analysis of leave-on samples[a] (%,w/w x 10^{-4})[b]

	BM	ML1	ML2	ML3	MC1	MC2	MC3	SB	HC	AC	AS	De1	De2	De3	SC	HNC1	HNC2	HF	HG
Plasticizers																			
DMA							0.0394												
DMP									0.0904								0.374		
DEP			4.96	104			0.840	5.00	6.53		357	3.51		1.15	0.665			1.78	
DIBP			0.930					1.24	3.16						0.987				
DBP		0.518	9.63					0.717	141		1.15				0.557			37.7	
DMEP												12.6					0.406		
DIPP																	0.0445		
DPP															0.447				
BBP			0.0616						0.472										
DEHA		0.317							0.669	0.391			0.135		0.913	0.387	7.34	0.857	
DEHP		0.703		0.676			0.578	25.8	0.809	0.647					2.76				
DPhP						2.36			0.499										
DOP													0.0842						
DINP								7.81			1.97								
Musks																			
Cashmeran		17.5									422			180					
Celestolide		3.34	0.0391	0.164	0.498		0.0120								2.35				
Phantolide		0.279		0.0329							0.546	5.48				0.767		0.283	
M. Ambrette				0.973			0.776									0.0443			
Traseolide	0.410		<LOQ								0.102	0.0809							
Galaxolide		998	0.862	146	283				0.166	0.624	0.179			0.0440	3640	1220		0.0573	
M. Xylene	0.0339															299	0.212		
Tonalide			0.318	27.0					0.0435	0.201		228	1960	0.0135		1100		0.0160	123	4.39
M. Tibetene																		0.0275	
Ambrettolide		84.0								2.68		210			0.0105				
M. Ketone																			6.21
DEHPd4[c]		103	114	118	105	97	105	106	109	112	99.5	79.7	68.9	98.0	107	113	93.9	82.7	63.3	71.2

[a] BM: body milk, ML: moisturizing lotion, MC: moisturizing cream, SB: sunblock, HC: hands cream, AC: anti aging cream, AS: aftershave, De: deodorant, SC: Straightening cream,HNC: hair nutrition cream, HF: hair foam, HG: hair gel. [b] Equivalent to μg g^{-1}. [c] Surrogate recovery (%). Blank spaces: below LOD. Grey cells: forbidden compounds (EC No 1223/2009)

3.3.2. Musks

In the case of rinse-off samples, galaxolide was found in all samples although the levels were low. Tonalide was found in most samples at low levels excluding a shampoo that contained almost a 0.2 % of this compound (1760 μg g^{-1}). Phantolide and traseolide were found in one sample each (6.5 and 0.4 μg g^{-1}) and one of the banned nitro musks, musk ambrette, excluded for use in cosmetic products since 1995, was found in a body scrub (0.4 μg g^{-1}).

Regarding the leave-on samples, tonalide and galaxolide were the most abundant musks (11 of the 19 samples), and the concentration of these fragrances was very high in several samples, with values about 0.1 % (1000 μg g^{-1}). Other polycyclic musks, celestolide, phantolide, and traseolide were found in 4 to 6 samples, and cashmeran was found in only three of the samples but at higher levels (up to 422 μg g^{-1}). The macrocyclic musk ambrettolide was also found in four samples, in two of them at high concentration (84 and 210 μg g^{-1}). Regarding nitro musks, musk ketone was found in one sample (6.2 μg g^{-1}). Two banned nitro musks, musk ambrette and musk tibetene, were also detected in 3 and 1 leave-on products, respectively, at low levels.

None of the musks neither the phthalates were included in the list of ingredients of the products. Although all products included the generic term “fragrance” or “parfum” in the label. As commented in the introduction, this term usually represents a complex mixture of chemicals, frequently dominated by the synthetic musks. Nevertheless, the presence of other compounds such DEP could be “hidden” under this word. Anyhow, we believe that manufactures should declare the presence of authorized phthalates on the product label; obviously, the banned substances should not be present in the product.

4. CONCLUSIONS

MSPD has been successfully applied to the determination of 18 plasticizers and 12 musk fragrances in leave-on and rinse-off cosmetics. These two families of compounds are extensively used by the cosmetic industry and subjected to restrictions according international regulations. To our knowledge, this study constitutes the first application of MSPD to the analysis of these families of compounds in cosmetics. Multivariate optimization was carried out using real cosmetic samples and method quality parameters were also evaluated on real cosmetic samples. The MSPD method was miniaturized and customized to avoid or minimize risks of phthalate contamination and to reduce residues and costs. It does not require special equipment since the extraction is performed in glass Pasteur pipettes with glass wool plugs and, thus, it can be easily implemented in any laboratory at negligible costs. Method accuracy and precision were satisfactory, showing mean recovery values from 85 to 105 %, and RSD was generally below 8 %. The method was also applied to a broad range of cosmetics demonstrating the suitability of the optimized procedure. Twenty-five out of thirty targets were detected in the samples. The presence of EU regulation prohibited phthalates (mainly DBP and DEHP, at concentrations up to 141 μg g^{-1}) and nitromusk was confirmed in a high number of cosmetics. None of the phthalates neither the musks were included in the list of ingredients of the products.

Acknowledgements

This research was supported by FEDER funds and projects CTQ2010-19831 (Ministerio de Ciencia e Innovacion, Spain), and CN 2012/299 (Consolidated Research Groups Program, Xunta de Galicia). L.S-P. and J.P.L. acknowledge Xunta de Galicia for a postdoctoral Angeles Alvariño, and Isabel Barreto contracts, respectively.

References

[1] A.Chisvert, A. Salvador, 6.1. Perfumes in cosmetics. Regulatory aspects and analytical methods for fragance ingredients and other related chemicals in cosmetics, in: A. Salvador, A. Chisvert (Eds.), Analysis of Cosmetic Products, Elsevier, Amsterdam, 2007, p.249.

[2] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products (recast), Off. J. Eur. Union L342/59 (2009).

[3] www.fda.gov/Cosmetics/ProductandIngredientSafety/SelectedCosmeticIngredients/ucm12825.htm (accessed 18.01.13).

[4] M. Llompart, C. Garcia-Jares, P. Landin, in: L.M.L Nollet (Ed), Chromatographic Analysis of the Environment, CRC Press, Taylor and Francis Group, Boca Raton, 2006, p. 1103.

[5] M.K. Stanley, K.A. Robillard, C.A. Staples, in: C.A. Staples (Ed.), The Handbook of Environmental Chemistry. Part Q. Phthalate Esters, vol. 3, Springer-Verlag, Berlin, 2003, p.1.

[6] D. Koniecki, R. Wang, R.P. Moody, J. Zhu, Environ. Res. 111 (2011) 329.

[7] G.G. Rimkus. The Handbook of Environmental Chemistry. Part X. Synthetic Musk Fragrances in the Environment, Vol. 3, Rimkus, G.G., Ed., Springer-Verlag, Berlin (2004).

[8] G.G. Rimkus, Toxicol. Lett. 111 (1999) 37.

[9] J.L. Reiner, K. Kannan, Chemosphere 62 (2006) 867.

[10] R.J.B. Peters, TNO-report R&I-A R 2005/011, TNO Environment and Geosciences, 2005.

[11] Commission of the European Communities Brussels, 30.11.2007 Commission Staff Working Document on the Implementation of the Community Strategy for Endocrine Disrupters. A range of substances suspected of interfering with the hormone systems of humans and wildlife (COM (1999) 706), (COM (2001) 262) and (SEC (2004) 1372).

[12] A.M. Peck, Anal. Bioanal. Chem. 386 (2006) 907.

[13] K. Bester, J. Chromatogr. A 1216 (2009) 470.

[14] F. David, P. Sandra, B. Tienpont, F. Vanwalleghem, M. Ikonomou, in: C.A. Staples (Ed.), The Handbook of Environmental Chemistry. Part Q. Phthalate Esters, vol. 3, Springer-Verlag, Berlin, 2003, p.9.

[15] M.R. Hadjmohammadi, E. Ranjbari, Int. J. Environ. Anal. Chem. 92 (2012) 1312.

[16] V. Matamoros, E. Jover, J.M. Bayona, Anal. Chem 82 (2010) 699.

[17] L. Yan, T. Yuan, H. Jiangyong, W. Wenhua, Anal. Sci. 25 (2009) 1125.

[18] M. Llompart, C. Garcia-Jares, C. Salgado, M. Polo, R. Cela. J. Chromatogr. A 999 (2003) 185.

[19] S. Machado, C. Gonçalves, E. Cunha, A. Guimaraes, M.F Alpendurada, Talanta 84 (2011) 1133.

[20] K. Luks-Betlej, P. Popp, B. Janoszka, H. Paschke, J. Chromatogr. A 938 (2001) 93.

[21] M. Polo, C. Garcia-Jares, M. Llompart, R. Cela, Anal. Bioanal. Chem. 388 (2007) 1789.

[22] G. Basaglia, M. C. Pietrogrande, Chromatographia 75 (2012) 361.

[23] A. Peñalver, E. Pocurull, F. Borrull, R.M. Marce, J. Chromatogr. A 872 (2000) 191.

[24] H. Farahani, M.R. Ganjali, R. Dinarvand, P. Norouzi, Talanta 76 (2008) 718.

[25] E. Psillakis, N. Kalogerakis, J. Chromatogr. A 999 (2003) 145.

[26] R. M Silva, J. M. F. Nogueira, Anal. Bioanal. Chem 396 (2010) 1853.

[27] N. Ramirez, R.M. Marce, F. Borrul, J. Chromatogr. A 1218 (2011) 156.

[28] J. Regueiro, M. Llompart, C. Garcia-Jares, C. Garcia-Monteagudo, R. Cela, J. Chromatogr. A 1190 (2008) 27.

[29] F. Kamarei, H. Ebrahimzadeh, Y. Yamini, Microchem. J.99 (2011) 26.

[30] L. Sanchez-Prado, C. Garcia-Jares, M. Llompart, J. Chromatogr. A 1217 (2010) 2390.

[31] M.L. Svoboda, M. Lewina, J.J. Yang, P. Falletta, H. Lee, Water Qual. Res. J. Can. 42 (2007) 11.

[32] H. Xiangang, Z. Qixing, Chromatographia 74 (2011) 489.

[33] L. Vallecillos, F. Borrull, E. Pocurull, J. Sep. Sci. 35 (2012) 2735.

[34] L. Sanchez-Prado, J.P. Lamas, C. Garcia-Jares, M. Llompart, Chromatographia 75 (2012) 1039.

[35] H. Chen, C. Wang, X. Xing, N. Hao, J. Liu, Int. J. Cosmet. Sci. 27 (2005) 205.

[36] D. De Orsi, L. Gagliardi, R. Porra, S. Berri, P. Chimenti, A. Granses, I. Carpani, D. Tonelli, Anal. Chim. Acta 555 (2006) 238.

[37] H.Y. Shen, H.L. Jiang, H.L. Mao, G. Pan, L. Zhou, J. Sep. Sci. 30 (2007) 48.

[38] L. Sanchez-Prado, M. Llompart, J.P. Lamas, C. Garcia-Jares, M. Lores, Talanta 85 (2011) 370.

[39] M. Lopez-Nogueroles, J.L. Benede, A. Chisvert, A. Salvador, Anal. Methods. 5 (2013) 409.

[40] L. Sanchez-Prado, G. Alvarez-Rivera, M. Lores, C. Garcia-Jares, J.P. Lamas, Anal. Bioanal. Chem. 401 (2011) 3293.

[41] L. Sanchez-Prado, J.P. Lamas, G. Alvarez-Rivera, M. Lores, C. Garcia-Jares, M. Llompart. J. Chromatogr. A 1218 (2011) 5055.

[42] G. Alvarez-Rivera, T. Dagnac, M. Lores, C. Garcia-Jares, L. Sanchez-Prado, J.P. Lamas, M. Llompart. J. Chromatogr. A 1270 (2012) 41.

[43] M. Polo, M. Llompart, C. Garcia-Jares, R. Cela, J. Chromatogr. A 1272 (2005) 63.

[44] L. Sanchez-Prado, G. Alvarez-Rivera, J.P. Lamas, M. Llompart, M. Lores, C. Garcia-Jares, Anal. Methods 5 (2013) 416.

[45] A.J.C Hubinger, D.C Donald, J. Cosmet. Sci. 57 (2006) 127.

1.2. DEVELOPMENT OF A MULTIANALYTE METHOD BASED ON MICRO-MATRIX-SOLID-PHASE DISERSION FOR THE ANALYSIS OF FRAGRANCE ALLERGENS AND PRESERVATIVES IN PERSONAL CARE PRODUCTS

Journal of Chromatography A (ed. Elsevier, Netherlands)
ISSN: 0021-9673

***J. Chromatogr. A., 1344 (2014) 1-14*. doi: 10.1016/j.chroma.2014.03.070**

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DEVELOPMENT OF A MULTIANALYTE METHOD BASED ON MICRO-MATRIX-SOLID-PHASE DISPERSION FOR THE ANALYSIS OF FRAGRANCE ALLERGENS AND PRESERVATIVES IN PERSONAL CARE PRODUCTS

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ABSTRACT

An effective, simple and low cost sample preparation method based on matrix solid-phase dispersion (MSPD) followed by gas chromatography–mass spectrometry (GC-MS) or gas chromatography-triple quadrupole-mass spectrometry (GC-MS/MS) has been developed for the rapid simultaneous determination of 38 cosmetic ingredients, 25 fragrance allergens and 13 preservatives. All target substances are frequently used in cosmetics and personal care products and they are subjected to use restrictions or labelling requirements according to the EU Cosmetic Directive. The extraction procedure was optimized on real non-spiked rinse-off and leave-on cosmetic products by means of experimental designs. The final miniaturized process required the use of only 0.1 g of sample and 1 mL of organic solvent, obtaining a final extract ready for analysis. The micro-MSPD method was validated showing satisfactory performance by GC-MS and GC-MS/MS analysis. The use of GC coupled to triple quadrupole mass detection allowed to reach very low detection limits (low ng g^{-1}) improving, at the same time, method selectivity. In an attempt to improve the chromatographic analysis of preservatives, the inclusion of a derivatization step was also assessed. The proposed method was applied to a broad range of cosmetics and personal care products (shampoos, body milk, moisturizing milk, toothpaste, hand creams, gloss lipstick, sunblock, deodorants and liquid soaps among others), demonstrating the extended use of these substances. The concentration levels were ranging from the sub parts per million to the parts per mill. The number of target fragrance allergens per samples was quite high (up to sixteen). Several fragrances (linalool, farnesol, hexylcinnamal, and benzyl benzoate) have been detected at levels >0.1% (1000 μg g^{-1}). As regards preservatives, phenoxyethanol was the most frequently found additive reaching quite high concentration (>1500 μg g^{-1}) in five cosmetic products. BHT was detected in eight samples, in two of them (a baby care product and a lipstick) at high concentrations (>1000 μg g^{-1}). Methyl paraben was also found at high levels (>1700 μg g^{-1}) in three leave-on samples. Finally, triclosan was found at the maximum concentration limit (0.3 %) laid down by the European regulation in two deodorant samples, and the total paraben concentration was close to the maximum concentration permitted (0.8 %) in one leave-on sample (body milk).

Keywords: fragrance allergens; preservatives; cosmetics; matrix solid-phase dispersion; personal care products; GC-MS/MS

1. INTRODUCTION

The majority of cosmetics and personal care products contain fragrances and preservatives. Fragrances are the most frequent causes of contact allergy to cosmetics. Allergic reactions and side effects include skin sensitivity, dermatitis, asthma attacks and migraine [1,2]. In order to reduce the risk of skin sensitization, the European legislation [3] requires monitoring 26 volatile compounds used in cosmetics the so-called potentially allergen substances (PAS) or fragrance allergens. Their presence must be indicated in the list of ingredients when their concentrations exceed 0.01% for rinse-off products (e.g. shampoos), and 0.001% for leave-on products (e.g lotions, deodorants). Of these 26 substances, 24 are chemically defined volatile compounds whereas the other two are natural moss extracts and do not correspond to defined chemicals [4]. Methyleugenol should also be considered for controlling because recent changes in EU regulations include the transfer of this compound to the Annex II (list of substances prohibited in cosmetic products) to the Annex III (list of substances which cosmetic products must not contain except subject to restrictions) [5].

Preservatives are used in cosmetic formulations to protect them against microbial growth, both to maintain product integrity and to care for consumers [6]. Parabens (alkyl esters of p-hydroxybenzoic acid) are the most widely used preservatives in cosmetic products, due to their broad antimicrobial spectrum, relatively low toxicity, and cost [7]. The estimated paraben exposure per person is 77.5 mg day^{-1} (50 mg via cosmetics and personal care products) [8]. European legislation restricts the preservation of cosmetic products by parabens to a maximum authorized concentration of 0.4% for a single ester or 0.8% for ester mixtures (w/w, calculated as acid) [3]. Although these compounds are not mutagenic agents, recent studies have reported that certain parabens have been associated with genotoxicity, allergies and may also act as antiandrogens [9-11]. Iodopropynyl butylcarbamate (IPBC), 2,4,4'-trichloro-2'-hydroxydiphenyl ether (triclosan) and bromine-containing preservatives such as bronidox are also included in a wide variety of cosmetics and personal care products to prevent or retard bacterial growth. Phenoxyethanol is increasingly turning up in cosmetics as a preservative and as an alternative to parabens; this glycol ether is used as an anti-bacterial as well as perfume stabilizer. Butyl hydroxytoluene (BHT) is frequently used in cosmetics as antioxidant.

In order to guarantee product safety according to regulations and to assess the health risk from the potential exposures, the development of analytical methods for the determination of fragrances and preservatives in cosmetic formulations is mandatory. Moreover, personal care products (PCPs) are included as "emerging organic contaminants" and significant amounts of these products and their metabolites can be present in the environment [12]. The increasing interest on the determination of the levels of both families of these compounds is reflected by the number of recent studies on this matter.

Several analytical methods have been developed for the determination of fragrance allergens in environmental samples, indoor air, and scented toys. In this way, dispersive liquid-liquid microextraction [13], ultrasound-assisted emulsification-microextraction and solid-phase microextraction (SPME) [14,15] have been employed to determine fragrance suspected allergens in natural waters, swimming pool waters, baby bathwaters and waste waters. These volatile substances have been analyzed in indoor air using SPME and solid-phase extraction (SPE) [16,17]. To determine fragrance allergens in scented toys methods using dynamic headspace and SPME

followed by GC-MS [18,19] have been reported. Regarding the analysis of fragrances allergens in cosmetics and personal care products, the scientific literature is scarce although some advanced extraction techniques such as pressurized liquid extraction (PLE) [20], and full evaporation dynamic headspace [21], have been used.

Analytical methodologies have also been reported for the determination of preservatives, mostly parabens, in water samples and biological matrices. The extraction of these compounds have mainly been carried out by SPE [22] but in last years, miniaturized techniques such as SPME [23] and hollow fibre liquid-phase microextraction [24] have been employed to determine parabens and triclosan in waters. Dispersive liquid-liquid microextraction has been proposed to determine parabens in human serum samples [25]. For the determination of preservatives in cosmetics solvent extraction using ultrasonic energy [26] and methods based on PLE [27] have been recently published.

Matrix solid-phase dispersion (MSPD) is as a very suitable analytical tool for the extraction of preservatives and fragrances from cosmetics, providing efficient and low cost extractions [28-30]. A miniaturized MSPD method has been recently proposed for the determination of plasticizers and synthetic musks in cosmetics [31].

Detection of allergen fragrances and preservatives has mainly been carried out by GC-MS. However, the complexity of the cosmetic matrices which contain many ingredients with similar chemical structures (common ions) can reduce analysis selectivity and reliability. The analysis of allergen fragrances is especially complex due to the difficulties to avoid co-elutions and the similarities in mass spectra [32]. Chaintreau et al. have proposed a chromatographic method based on the use of two different polarity capillary columns together with the use of three ions per fragrance to solve co-elution problems [33]. Tandem mass spectrometry (MS/MS) in combination with GC is a valuable approach that improves selectivity and analyte sensitivity, minimising or even avoiding most of matrix interferences. Recently, Qving et al. [34] described the determination of 48 fragrance allergens in toys using GC coupled to ion trap MS/MS demonstrating that the application of MS/MS is an accurate and effective technique for the analysis of fragrance allergens in matrices composed of complex components. To the best of our knowledge, there are not references on the analysis of fragrance allergens in cosmetics or personal care products by GC-MS/MS, and only one reference that includes the determination of only four parabens [35].

The aim of the present study is the development of a MSPD combined with GC-MS and GC-MS/MS method for the simultaneous determination of 25 fragrance allergens and 13 preservatives in cosmetics and personal care products. Miniaturization of the extraction technique will be studied with the purpose of minimizing the consumption of sample, reagents and solvents, meeting “green chemistry” requirements. GC-MS and GC-MS/MS will be compared to determine whether the use of tandem mass spectrometry leads to an improvement in the determination of the target compounds in rinse-off and leave-on cosmetic samples attending both sensitivity and selectivity.

2. EXPERIMENTAL

2.1. Chemicals, materials and samples

The studied compounds, CAS numbers, suppliers, purity of the standards and restrictions according European legislation are summarized in **Table 1**. Some of the allergen fragrances are commercialized and were acquired as mix of isomers (see **Table 1**). Deuterated methyl-4-hydroxybenzoate-2,3,5,6-d_4 (MeP_d_4; 98atom% D) and benzyl_d_7 alcohol (98atom% D) used as surrogate standard, were obtained from C/D/N Isotopes (Quebec, Canada) and Aldrich (St. Louis, MO, USA), respectively.

Acetone, ethyl acetate and n-hexane were provided by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Florisil (60-100 mesh) was purchased from Supelco Analytical (Bellefonte, PA, USA) and anhydrous sodium sulphate (99 %) from Panreac (Barcelona, Spain).

Individual stock solutions of each compound were prepared in acetone. Further dilutions and mixtures were prepared in acetone, hexane or ethyl acetate. All solutions were stored in amber glass vials at -20°C. All solvents and reagents were of analytical grade.

All the glass, metallic and ceramic materials, the sorbents (Florisil and sodium sulphate), and the glass wool for laboratory use (Sigma-Aldrich) were baked at 230°C for 12 h before use to eliminate possible contamination. All materials were allowed to cool down wrapped with aluminum foil. Florisil and sodium sulphate were allowed to cool down in a desiccator.

Cosmetic samples from national and international brands were purchased from local sources. They included leave-on and rinse-off products such as body milk, moisturizing milk, nail polish remover, toothpaste, hand cream, lipstick, gloss lipstick, sunblock, deodorants, shampoos and liquid soaps, among others. Samples were kept in their original containers at room temperature until their analysis.

Table 1. Target compounds: chemical names, suppliers, purity, CAS and EU restrictions (EC No 1223/2009).

Key	*Fragrance allergens*	*Chemical names*	*Purity (%)*	*CAS*	*Maximum concentration permitted* [3]
1	Limonene[e]	(4R)-1-Methyl-4-(1-methylethenyl)cyclohexene	97[b]	5989-27-5	n.r
2	Benzyl alcohol[e]	Benzene methanol	99[c]	100-51-6	1% (as preservative)
3	Linalool[e]	3,7-Dimethyl-1,6-octadien-3-ol	97[b]	78-70-6	n.r
4	Methyl-2-octynoate[e]	Methyl heptin carbonate	≥99[d]	111-12-6	n.r
5	Citronellol[e]	(±)-3,7-Dimethyoct-6-en-1-ol	95[b]	106-22-9	n.r
6	Citral [e]	3,7-Dimethyl-2,6-octadienal	95[b]	5392-40-5	n.r
7	Geraniol[e]	3,7-Dimethyl-(2E)-2,6-octadien-1-ol	≥96[a]	106-24-1	n.r
8	Cinnamal[e]	3-Phenyl-2-propenal	≥93[d]	104-55-2	n.r
9	Hydroxycitronellal[e]	7-Hydroxy-3,7-dimethyloctanal	≥95[d]	107-75-5	1%
10	Anise alcohol[e]	4-Methoxybenzyl alcohol	98[b]	105-13-5	n.r
11	Cinnamyl alcohol[e]	3-Phenyl-2-propen-1-ol	98[b]	104-54-1	n.r
12	Eugenol[e]	2-Methoxy-4-(2-propenyl)-phenol	99[b]	97-53-0	n.r
13	Methyleugenol[e]	1,2-Dimethoxy-4-(2-propenyl)-benzene	99[b]	93-15-2	0.01% (fine fragrance); 0.004% (eau de toilette); 0.002% (fragrance cream); 0.0002% (other leave-on products); 0.001% (rinse-off products)
14	Isoeugenol[e]	2-Methoxy-4-(1-propenyl)phenol	98[b]	97-54-1	0.02%
15	Coumarin[e]	2H-1-benzopyran-2-one	99[b]	91-64-5	n.r

Table 1. Continuation

16	α-isomethyl ionone[e]	3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1yl)-3-buten-2-one	≥85[a]	127-51-5	n.r
17	Lilial[e]	2-(4-*tert*-Butylbenzyl)propionaldehyde	≥85[a]	80-54-6	n.r
18	Amyl cinnamal[e]	2-Benzylideneheptanal	97[b]	122-40-7	n.r
19	Lyral[e]	Hydroxyhexyl-3-cyclohexene carboxaldehyde	≥97[a]	31906-04-4	n.r
20	Amylcinnamyl alcohol[e]	2-Pentyl-3-phenylprop-2-en-1-ol	≥85[a]	101-85-9	n.r
21	Farnesol[e]	3,7,11-trimethyldodeca-2,6,10-trien-1-ol	95[b]	4602-84-0	n.r
22	Hexylcinnamal[e]	2-Benzylideneoctanal	≥95[d]	101-86-0	n.r
23	Benzyl benzoate[e]	Phenylmethyl benzoate	98[c]	120-51-4	n.r
24	Benzyl salicylate[e]	Benzyl-2-hydroxybenzoate	≥99[a]	118-58-1	n.r
25	Benzyl cinnamate[e]	3-Phenyl-2-propenoic acid phenylmethyl ester	99[b]	103-41-3	n.r
Key	***Preservatives***	***Chemical names***	***Purity (%)***	***CAS***	***Maximum concentration permitted* [3]**
26	Bronidox	5-Bromo-5-nitro-1,3-dioxane	≥99[a]	30007-47-7	0.1%
27	Phenoxyethanol (phEtOH)	2-Phenoxyethanol	99[a]	122-99-6	1%
28	Methyl paraben (MeP)	Methyl 4-hydroxibenzoate	99[a]	99-76-3	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
29	BHA	Butylated hidroxyanisole	98.5[a]	25013-16-5	n.r
30	BHT	Butylated hydroxytoluene	99[a]	128-37-0	n.r
31	Ethyl paraben (EtP)	Ethyl 4-hydroxybenzoate	99[a]	120-47-8	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
32	Isopropyl paraben (iPrP)	Isopropyl 4-hydroxybenzoate	≥99[d]	4191-73-5	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
33	Propyl paraben (PrP)	Propyl 4-hydroxybenzoate	99[a]	94-13-3	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
34	IPBC	Carbamic acid, butyl-3-iodo-2-propynyl ester	97[a]	55406-53-6	0.02% (rinse-off products); 0.01% (leave-on products); 0.0075% (deodorants)
35	Isobutyl paraben (iBuP)	Isobutyl 4-hydroxybenzoate	≥97[d]	4247-02-3	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
36	Butyl paraben (BuP)	Butyl 4-hydroxybenzoate	99[a]	94-26-8	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
37	Triclosan	2,4,4'-Trichloro-2'-hydroxydiphenyl ether	≥97[a]	3380-34-5	0.3%
38	Benzyl paraben (BzP)	Benzyl hydroxybenzoate	99[b]	94-18-8	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)

[a]Fluka Chemie GmbH (Steinheim, Germany). [b]Sigma Aldrich Chemie GmbH (Steinheim, Germany). [c]Chem Service (West Chester, USA). [d]SAFC Supply Solutions (St. Louis, USA). [e]The presence of the substance must be indicated in the list of ingredients when its concentration exceeds 0.001% (leave-on products) and 0.01% (rinse-off products). n.r: no restricted by EC No 1223/2009. Numbers corresponding with target compounds in Figure 1.

2.2. MSPD procedure

0.1 grams of cosmetic were exactly weighted into a 10-mL glass vial and spiked with 10 µL of benzyl alcohol-d_7 and MeP-d_4 surrogate solution (25 µg mL^{-1}). When it was necessary, the sample was spiked with 10 µL of the corresponding acetone solution of the target compounds to get the

desired final concentration. Then, the sample was gently blended with 0.2 g of a drying agent (anhydrous Na_2SO_4) and 0.4 g of dispersing sorbent (Florisil or sand) into a porcelain mortar using a porcelain pestle until a homogeneous mixture was obtained (ca. 5 min). The mixture was transferred into a glass Pasteur pipette (aprox. 150 mm), with a small amount of glass wool at the bottom, containing 0.1 g of Florisil (to obtain a further degree of fractionation and sample clean-up). Finally, a small amount of glass wool was placed on top of the sample before compression with a small metallic spatula. Elution was made by gravity flow with ethyl acetate or hexane:acetone (1:1, v/v), collecting 1 or 2 mL of extract into a volumetric flask. The MSPD extracts, diluted when necessary, were directly analyzed by GC-MS and GC-MS/MS.

2.3. Derivatization procedure

Acetylation was carried out by adding 50 µL of acetic anhydride containing 2.5% of pyridine to 0.5 mL of the standard or extract solutions. The mixture was maintained at 80°C for 15 min. The derivatized MSPD extracts, diluted when necessary, were directly analyzed by GC-MS and GC-MS/MS. Optimization of the derivatization conditions to improve the chromatographic analysis of phenolic preservatives was optimized elsewhere [26,27]. Three of the studied compounds (bronidox, BHT and IPBC) did not undergo derivatization. Bronidox and IPBC do not have chemical groups susceptible to acetylation; the acetylation of BHT could not be demonstrated as the highly hindered hydroxyl group with poor nucleophilicity may prevent the acetylation under the studied conditions. For the other preservatives and antioxidants, reaction yield was quantitative and satisfactory.

2.4. GC-MS analysis

The GC–MS analysis was performed using an Agilent 7890A (GC)-Agilent 5975C inert MSD with triple axis detector and an Agilent 7693 autosampler from Agilent Technologies (Palo Alto, CA, USA). The temperatures of the transfer line, the quadrupole and the ion source were set at 290, 150 and 230°C, respectively. The system was operated by Agilent MSD ChemStation E.02.00.493 software.

Separation was carried out on a SLB^{TM}-5ms capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness) obtained from Supelco Analytical (Bellefonte, PA, USA). Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 mL min^{-1}. The GC oven temperature was programmed from 60°C (held 1 min) to 100°C at 8°C min^{-1}, to 150°C at 20°C min^{-1}, to 200°C at 25°C min^{-1} to 220°C at 8°C min^{-1} and 30°C min^{-1} to 290°C (held 15 min). For the analysis of the derivatized extracts, the GC-MS oven temperature was programmed from 80°C (held 2 min) to 290°C at 14°C min^{-1}. Pulsed splitless mode was used for injection (30 psi, held 1.2 min). After 1 min, the split valve was opened (75 mL min^{-1}), and the injector temperature was kept at 260°C. The injection volume was 1 µL. The electron multiplier was set at a nominal value of 1376 V.

2.5. GC-MS/MS analysis

The GC–MS/MS analysis was performed using a Thermo Trace 1310-Thermo Triple Quadrupole TSQ 8000 with autosampler IL 1310 from Thermo Scientific (San Jose, CA, USA). The temperatures of the transfer line and the ion source were set at 290 and 350°C, respectively. The system was operated by Xcalibur 2.2 and Trace Finder TM 3.0.

Separation was carried out on a TG-5 SILMS capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness) obtained from Thermo Scientific (San Jose, CA, USA). Helium (purity 99.999%) was

employed as carrier gas at a constant column flow of 1.0 mL min^{-1}. The GC oven temperature was programmed from 60°C (held 1 min) to 100°C at 8°C min^{-1}, to 150°C at 20°C min^{-1}, to 200°C at 25°C min^{-1}, to 220°C at 8°C min^{-1}, and 30°C min^{-1} to 290°C (held 9.5 min); and from 80°C (held 2 min) at 14°C min^{-1} to 290°C (held 3 min) for the derivatized extracts analysis. Splitless w/Surge mode was used for injection (200 kPa, held 1.2 min). After 1 min the split valve was opened (75 mL min^{-1}), and the injector temperature was kept at 260°C. The injection volume was 2 µL. The electron multiplier was set at a nominal value of 1638 V.

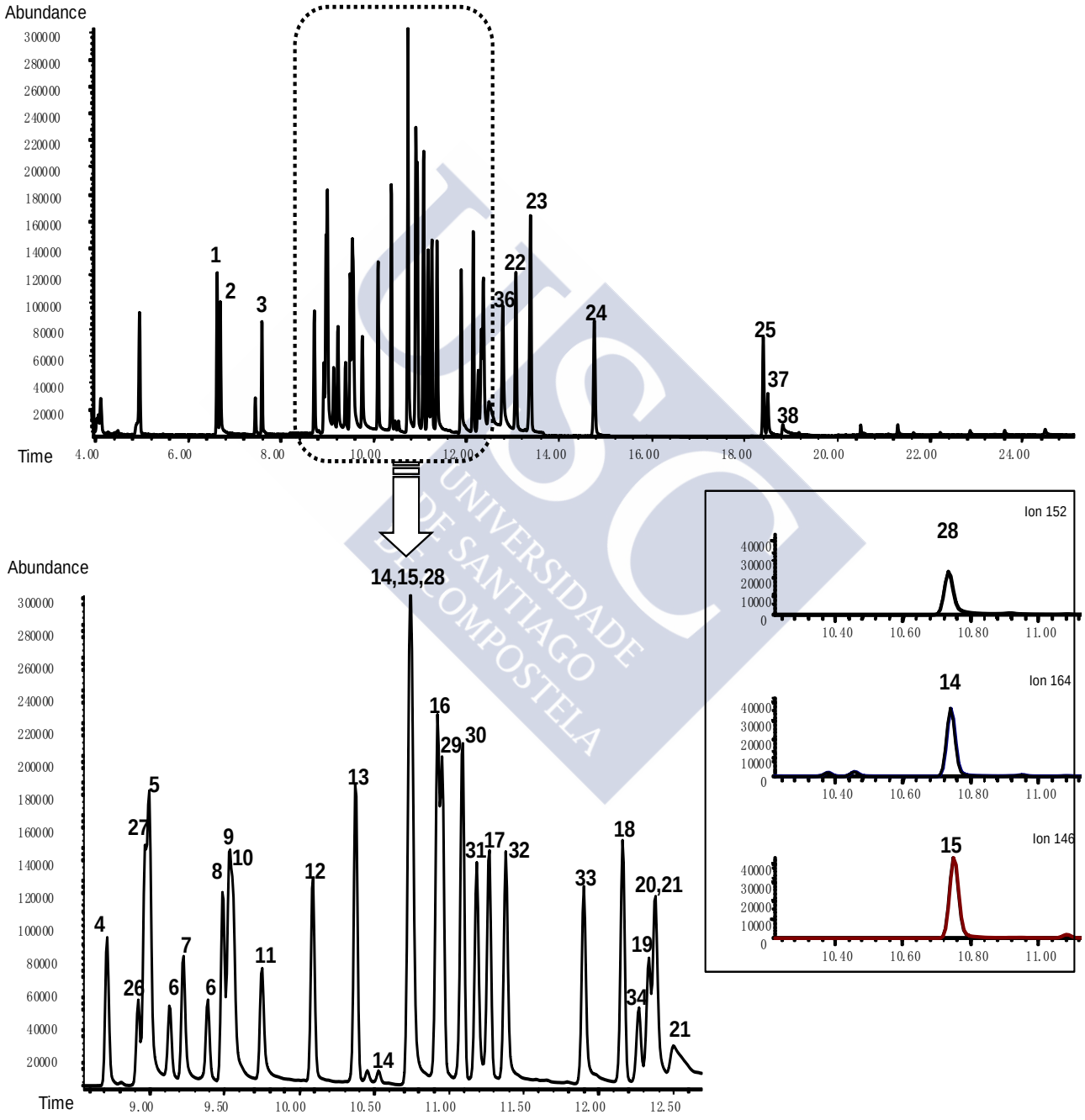

Fig. 1. Chromatogram of a 1000 ng mL^{-1} standard solution of all target analytes in ethyl acetate (see compound key in **Table 1**)

2.6. Statistical analysis

Basic and descriptive statistics, as well as experimental design analysis, were performed using Statgraphics-Plus v5.1 (Manugistics, Rockville, MD, USA) as software package. Experimental design was applied in the optimization of the extraction method to analyze the simultaneous effect of the experimental parameters affecting MSPD.

3. RESULTS AND DISCUSSION

The chromatographic conditions were optimized to achieve an adequate separation of the target compounds, 25 fragrance allergens and 13 preservatives (see conditions in the experimental section). For GC-MS analysis, the mass spectra detector (MSD) was operated in selected ion monitoring (SIM) mode, monitoring three ions per compound. For GC-MS/MS analysis, the detector was operated in selected reaction monitoring (SRM) mode selecting three transitions for each compound according to ion abundance. The most intense transition was used for quantification purposes, whereas the second and the third ones were employed for identification/confirmation purposes **(Tables S1 and S2). Figure 1** shows a chromatogram of a 1000 ng mL^{-1} standard solution in ethyl acetate. For some analytes constituted by isomeric mixtures two chromatographic peaks were obtained (citral, farnesol). As can be seen, some of the analytes co-elute. As previously mentioned in the introduction, the chromatographic separation of fragrance allergens is quite complex and some authors have propose the use of two different GC capillary columns and three ions (SIM mode) employing a total of six calibration curves per compound (specially designed software is required) [33]. In our case the coelution problems (e.g. isoeugenol, coumarin, MeP) could be initially overcome since none of the selected ions were common (see as exemple, the ion chromatograms in **Figure 1**). In addition, the use of MS/MS in the SRM mode improves selectivity by the use of specific transitions (three per compound), which could virtually eliminate matrix background.

3.1. MSPD Optimization

All the optimization studies have been developed employing non-spiked real samples. In this way, the real matrix-analyte interactions are considered in the selection of the most suitable extraction conditions. Since the objective was to develop a micro-MSPD method, only 0.1 g of sample were used in all experiments. The sample was mixed with the sample drying agent, anhydrous sodium sulphate (1:2, w/w). As described in the experimental section, all experiments were performed on 1.5 mL Pasteur pipettes with glass wool stoppers that were discarded after each extraction. Two factorial designs were carried out: for the first one, a leave-on cosmetic (moisturizing cream), and for the second one, a rinse-off cosmetic (shower gel), were employed. In both cases, the factors studied comprised the elution solvent, the dispersant, and the solvent volume **(Table 2)**.

Table 2. Factors and levels considered in the experimental designs

Factor	*Key*	*Lower level*	*Upper level*
Solvent	A	Hexane:acetone	Ethyl acetate
Dispersant	B	Sand	Florisil
Volume (mL)	C	1	2

3.1.1. Leave-on sample

The analysis of the sample showed the presence of 13 target analytes. All of them were included in the product ingredients label. In addition, two phthalates (DEP and DBP) and the musk fragrance galaxolide were found in the product.

The ANOVA results are summarized in **Table 3**. Factor B, the dispersant, was significant for the most volatile analytes (excluding limonene). The other factors, as well as the factor interactions, were no significant ($p>0.05$). **Figure 2** shows the Pareto charts and the main effect plots for some analytes, benzyl alcohol, geraniol, and phenoxyethanol, obtained in the factorial experiment design. In the Pareto charts, the standardized effects are plotted in decreasing order of absolute magnitude, thus making it easier to see which ones are the most important factors and interactions. In addition, the line drawn on the chart indicates whether an effect is statistically significant at a specified significance level (in this case, 95%). Main effect plots show how the response varies when each factor is changed from its low level to its high level, while all other factors are held at the center of the experimental domain. In the Pareto charts, factor B clearly exceeds the significance limit (vertical line in the graphs) for benzyl alcohol and geraniol and it is close to the line for phenoxyethanol whereas the other factors are no significant. The main effects plots show how higher response is achieved when using Florisil instead of sand. The other two factors, solvent and solvent volume do not affect the response obtained.

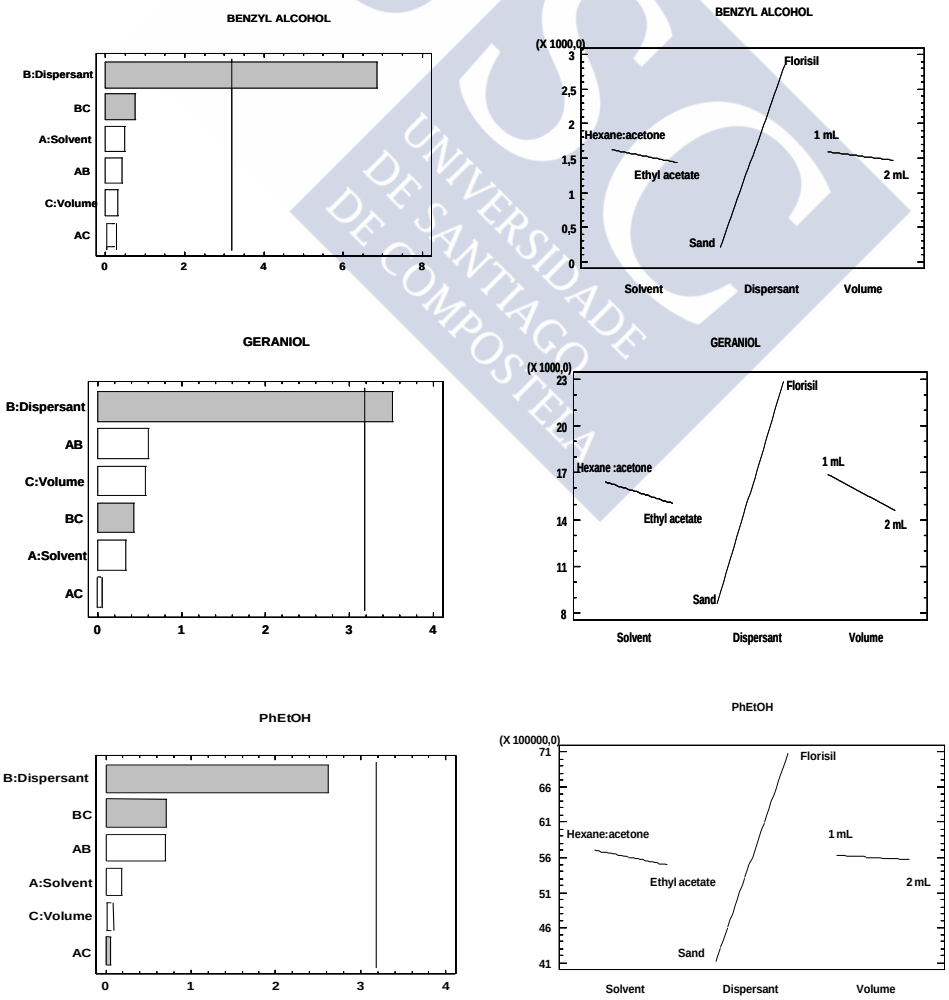

Fig. 2. Pareto charts and main effects plot showing the significant factors (95%) for the leave-on sample.

3.1.2. Rinse-off sample

The analysis of the sample showed the presence of limonene, linalool, citronellol, geraniol, eugenol, hexylcinnamal, benzyl benzoate and methyl paraben. The phthalates DEP and DBP were also found in this sample. The ANOVA study (**Table 3**) showed the statistical significance of factor B, the dispersant, for the fragrances (excluding limonene) and factor C, the elution volume, for many of the analytes. The Pareto charts for some analytes are included in **Figure 3**. Factors B and C are the most important with the highest standardized effect (larger bar). Also main effect diagrams are included; as can be seen, higher response is again achieved using Florisil instead of sand. Regarding the elution volume, the low level of the factor, 1 mL, was more favorable than 2 mL. Regarding the solvent, both gave similar response.

In view of the results, it is possible to establish a general method for the simultaneous extraction of the fragrance allergens and the preservatives both in leave-on and rinse-off cosmetics. The selected conditions involve the use of Florisil as dispersant and the elution with 1 mL of solvent. As the solvent was a non significant factor, both solvents, hexane:acetone and ethyl acetate, could be employed. In next studies, ethyl acetate was chosen.

Table 3. F ratios and p values obtained in the analysis of variance for the leave-on and rinse-off study[a].

Leave-on	**A: Solvent**		**B: Dispersant**		**C: Volume**		**AB**		**AC**		**BC**	
Compounds	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Limonene	0.06	0.82	3.68	0.15	0.10	0.77	0.06	0.82	0.42	0.56	0.10	0.77
Benzyl alcohol	0.23	0.66	**46.9**	**0.01**	0.10	0.77	0.17	0.70	0.03	0.88	0.55	0.51
Linalool	0.20	0.68	**41.2**	**0.01**	0.00	0.96	0.19	0.69	0.01	0.91	0.02	0.89
Geraniol	0.11	0.76	**12.4**	**0.03**	0.32	0.61	0.36	0.59	0.00	0.98	0.18	0.70
Ionone	0.12	0.75	8.70	0.06	1.03	0.38	0.53	0.51	0.02	0.89	0.30	0.62
Lilial®	0.03	0.87	2.13	0.24	0.69	0.46	0.56	0.50	0.05	0.84	0.28	0.63
Hexylcinnamal	0.02	0.89	0.29	0.62	0.51	0.52	0.60	0.49	0.02	0.89	0.31	0.61
DEP	1.29	0.33	6.86	0.07	0.28	0.63	3.85	0.14	0.84	0.42	0.04	0.85
DBP	1.21	0.35	1.17	0.35	0.32	0.61	0.72	0.45	0.02	0.89	0.71	0.46
PhEtOH	0.03	0.86	6.86	0.07	0.00	0.95	0.48	0.53	0.00	0.96	0.50	0.52
MeP	0.00	0.99	0.02	0.90	0.19	0.69	0.74	0.45	0.03	0.86	0.13	0.74
EP	0.00	0.97	0.00	0.97	0.50	0.53	0.79	0.43	0.03	0.86	0.11	0.75
PrP	0.01	0.92	0.00	0.97	0.97	0.39	0.80	0.43	0.04	0.86	0.16	0.71
IBuP	0.01	0.91	0.11	0.76	0.28	0.63	0.73	0.45	0.02	0.89	0.18	0.69
BuP	0.01	0.92	0.01	0.93	0.60	0.49	0.78	0.44	0.06	0.82	0.11	0.76
Galaxolide	0.03	0.88	0.18	0.69	0.42	0.56	0.64	0.48	0.03	0.86	0.28	0.63
Rinse-off	**A. Solvent**		**B: Dispersant**		**C: Volume**		**AB**		**AC**		**BC**	
Compounds	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**P**
Limonene	0.16	0.71	7.39	0.07	0,02	0.90	0.16	0.71	0.06	0.82	0.02	0.90
Linalool	0.06	0.82	**189**	**0.001**	4.65	0.12	2.44	0.21	0.02	0.88	1.44	0.31
Citronellol	0.04	0.85	**34.8**	**0.001**	9.55	0.05	1.53	0.30	0.12	0.75	0.07	0.80
Geraniol	0.01	0.91	**28.8**	**0.01**	**13.1**	**0.03**	1.64	0.29	0.01	0.91	0.08	0.79
Eugenol	0.38	0.58	**30.8**	**0.01**	**13.8**	**0.03**	0.79	0.44	0.07	0.81	0.90	0.41
Hexylcinnamal	1.64	0.29	**9.00**	**0.05**	4.42	0.12	0.44	0.55	0.25	0.64	2.34	0.22
Benzyl benzoate	11.9	0.05	5.04	0.11	**16.2**	**0.02**	4.89	0.11	10.1	0.05	3.39	0.16
DEP	2.33	0.22	1.48	0.31	**26.4**	**0.01**	1.39	0.32	2.75	0.19	1.10	0.37
DBP	2.48	0.21	0.70	0.46	6.37	0.08	0.21	0.67	0.50	0.53	1.50	0.30
MeP	0.12	0.75	6.89	0.07	**10.2**	**0.04**	0.53	0.51	3.95	0.14	0.18	0.70

[a] $p<0.05$ means statistical significance.

3.2. Derivatization

For the optimization study, all the analytes were determined in a single GC run. Nevertheless, the chromatographic peak shape as well as the chromatographic response can be improved for most preservatives including a derivatization step. Direct analysis produced peaks with appreciable tailing for some compounds due to the interaction of hydroxyl groups with the chromatographic system. Therefore, a derivatization step was introduced prior to GC determination to improve preservative chromatographic analysis. In this way, the MSPD extract was divided in two aliquots: one of them was directly analyzed and the other one was analyzed after derivatization. Acetylation with acetic anhydride is one of the most simple and cheap derivatization procedures for phenolic compounds. The procedure to obtain standard solutions of the corresponding acetylated compounds was based on previous work dealing with the acetylation of preservatives and other phenolic species [23,27], and it is described in the experimental section. Three of the preservatives (bronidox, IPBC, and BHT) did not undergo derivatization. For the other compounds, reaction yield was quantitative and satisfactory, improving significantly the chromatographic analysis of the target compounds. In any case, preservatives method validation has been evaluated in both cases, with and without the inclusion of an acetylation step. The acetylated derivatives were stable for at least several weeks.

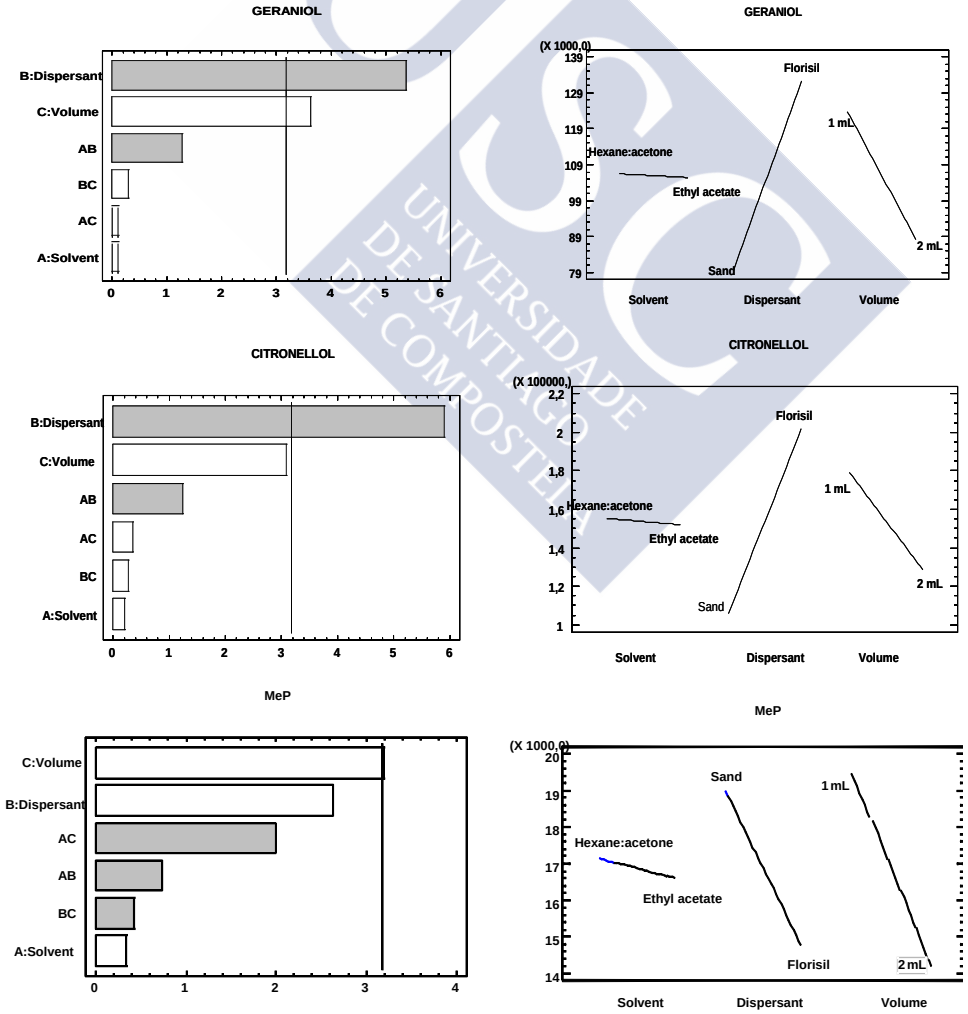

Fig. 3. Pareto charts and main effects plot showing the significant factors (95%) for the rinse-off sample.

3.3. Method performance

The GC-MS and GC-MS/MS method performance parameters for the 25 fragrance allergens and 13 preservatives (non derivatized) are summarized in **Tables 4 and 5** respectively. All 38 targets were simultaneously analyzed. Regarding the instrumental linearity, the method exhibited a direct proportional relationship between the amount of each analyte and the chromatographic response. Calibration standards in ethyl acetate were prepared covering a concentration range from 1 to 1000 ng mL^{-1}. Correlation coefficients R≥0.995 for GC-MS and GC-MS/MS were obtained in most cases. Method precision was studied within a day (n=5) and among days (n=8) at two concentration levels, 50 and 500 ng mL^{-1} (**Table S3**). RSD values ranged from 0.24-7.1% (intra-day precision) and 0.61-12% (inter-day precision) for GC-MS analysis; for GC-MS/MS analysis, RSD ranged from 0.56-7.1% and 1.8-13% (intra and inter-day precision, respectively). Instrumental detection limits (IDLs) were at the low ng mL^{-1} or below 1 ng mL^{-1} (GC-MS/MS); for many analytes, IDLs were between 4 and 10 times lower using GC-MS/MS.

Method quality parameters were evaluated using real cosmetic samples and are also shown in **Tables 4 and 5**. In this way, recovery studies were carried out by applying the optimized method to the extraction of two real samples spiked at 2, 10 and 20 μg g^{-1}: a rinse-off sample (liquid baby soap) and a leave-on sample (regenerating cream). These samples were expressly selected for recovery studies since they were almost free of the target fragrances and preservatives and so, specially suitable for recovery evaluation. The leave-on sample was labeled as perfume free and preservative-free. Additional recovery studies were also performed in other real samples (liquid soap, shower gel, shampoo, sunblock, body milk and lipstick) to demonstrate method suitability and the absence of matrix effects. The results are provided as supplementary material (**Table S4, S5**). Recoveries were calculated as the ratio between the detected amount and the spiked amount (x 100). For samples containing the analyte the initial amount in the sample was substrate from the total amount [35]. If the initial amount in the sample is higher than the spiked level recovery is not calculated since the error associated to the estimated value would be high. Recoveries were between 83 % and 115 % in both samples included in **Tables 4 and 6** at the three levels with the only exception of limonene. For this compound, slightly lower recoveries were obtained with a mean value of 73 %. This lower recovery could be probably due to the high volatility of this compound which could give analyte loss during MSPD treatment, especially during sample disruption. Recoveries were also satisfactory for the other seven samples tested (generally above 90 %, see **Table S4, S5**) with the exception of limonene with recoveries about 70 % in some cases. Since all samples were analyzed in triplicate, precision was also assessed (see RSD values in the tables) attaining RSD values generally lower than 10 %. Therefore, the method can be considered suitable for the determination of all target fragrance allergens and preservatives in real samples.

Limits of detection (LODs) were calculated as the compound concentration giving a signal-to-noise ratio of three (S/N=3). As shown in **Table 4**, LOD values for the fragrance allergens ranged from 0.00430 μg g^{-1} to 0.0600 μg g^{-1}, for GC-MS analysis (excluding farnesol). For GC-MS/MS analysis, LOD values ranged from 0.0004 to 0.025 μg g^{-1}. For preservatives (**Table 5**), LOD values were between 0.006-0.100 μg g^{-1} (GC-MS analysis) and 0.0015-0.037 μg g^{-1} (GC-MS/MS analysis).

Table 4. Quality parameters of the method for the GC-MS and GC-MS/MS analysis of fragrance allergens

	GC-MS (SIM)			GC-MS/MS (SRM)			Recoveries (% RSD)[a]					
	Correlation coefficient (R)	IDL (ng mL^{-1})	LOD[b] (%, w/w x10^4)	Correlation Coefficient (R)	IDL (ng mL^{-1})	LOD[b] (%, w/w x 10^4)	Rinse-off			Leave-on[c]		
Fragrances							2 µg g^{-1}	10 µg g^{-1}	20 µg g^{-1}	2 µg g^{-1}	10 µg g^{-1}	20 µg g^{-1}
Limonene	0.9993	1.07	0.0107	1.0000	0.13	0.00130	66.1 (8.0)	70.7 (2.1)	65.5 (1.7)	74.2 (7.4)	84.1 (0.53)	77.0 (8.8)
Benzyl Alcohol	0.9980	2.91	0.0291	0.9999	0.10	0.00100	112 (3.2)	106 (12)	106 (4.0)	112 (7.3)	105 (4.4)	112 (1.7)
Linalool	0.9984	2.10	0.0210	0.9999	0.60	0.00600	87.4 (5.8)	115 (6.0)	87.4 (2.7)	106 (1.1)	91.0 (7.6)	104 (2.5)
Methyl-2-octynoate	0.9969	2.83	0.0283	0.9999	0.62	0.00620	109 (1.1)	104 (6.1)	87.4 (12)	104 (1.2)	84.3 (6.1)	105 (2.5)
Citronellol	0.9965	2.97	0.0297	0.9999	2.07	0.0207	103 (1.7)	108 (4.8)	83.9 (2.3)	106 (9.8)	95.3 (7.6)	106 (1.7)
Citral	0.9973	2.83	0.0283	0.9997	2.12	0.0212	110 (2.9)	113 (8.6)	114 (6.6)	112 (1.1)	98.0 (2.3)	113 (2.4)
Geraniol	0.9969	2.98	0.0298	0.9995	2.51	0.0251	103 (5.0)	86.0 (5.1)	92.8 (6.2)	95.2 (9.1)	99.4 (5.4)	86.4 (3.0)
Cinnamal	0.9984	3.00	0.0300	0.9997	0.23	0.00230	112 (9.3)	98.0 (3.6)	88.1 (3.7)	105 (1.1)	91.8 (5.6)	102 (1.5)
Hydroxicitronellal	0.9972	2.01	0.0201	0.9999	0.51	0.00510	103 (2.4)	94.3 (5.4)	86.9 (5.1)	109 (8.7)	108 (3.1)	92.5 (2.3)
Anise Alcohol	0.9972	4.00	0.0400	0.9997	2.01	0.0201	114 (15)	88.1 (6.3)	89.2 (4.6)	115 (9.8)	96.0 (0.15)	106 (2.0)
Cinnamyl Alcohol	0.9965	5.08	0.0508	0.9994	1.91	0.0191	112 (13)	96.8 (13)	96.0 (7.1)	114 (2.8)	92.1 (7.7)	108 (2.1)
Eugenol	0.9964	2.99	0.0299	0.9990	0.56	0.00560	104 (4.1)	103 (2.6)	94.0 (5.0)	88.1 (9.9)	89.2 (7.5)	105 (2.2)
Methyleugenol	0.9982	2.00	0.0200	0.9998	0.06	0.000600	97.0 (2.7)	101 (2.6)	88.6 (3.2)	95.4 (8.2)	94.7 (8.2)	102 (2.0)
Isoeugenol	0.9976	2.93	0.0293	0.9987	1.70	0.0170	88.3 (8.3)	89.3 (2.3)	85.2 (4.6)	80.9 (1.3)	80.4 (9.3)	91 (2.9)
Coumarin	0.9997	1.70	0.0170	0.9998	0.25	0.00250	109 (2.4)	93.7 (3.3)	87.5 (2.7)	101 (1.1)	102 (0.94)	105 (1.7)
cis-isomethyl ionone	0.9985	0.81	0.00810	0.9998	0.05	0.000500	93.2 (4.6)	92.3 (5.6)	93.7 (3.3)	94.2 (13)	91.0 (3.3)	101 (1.5)
Lilial	0.9984	0.43	0.00430	0.9999	0.10	0.00100	90.4 (3.3)	83.0 (2.4)	96.5 (3.7)	93.1 (9.2)	91.4 (6.8)	103 (2.2)
Amyl Cinnamal	0.9963	2.04	0.0204	0.9995	1.60	0.0160	105 (5.6)	109 (1.7)	95.9 (3.5)	105 (9.1)	88.0 (6.3)	104 (1.6)
Lyral	0.9937	2.06	0.0206	0.9997	1.91	0.0191	97 (8.1)	115 (17)	100 (8.3)	110 (9.9)	95.2 (6.4)	103 (2.1)
Amylcinnamyl Alcohol	0.9930	6.00	0.0600	0.9989	1.50	0.0150	107 (6.7)	122 (4.5)	107 (5.3)	105 (9.3)	89.5 (10)	108 (2.2)
Farnesol	0.9978	70	0.700	0.9956	52	0.520	-	105 (15)	114 (14)	-	93.1 (12)	109 (4.3)
Hexylcinnamal	0.9954	2.99	0.0299	0.9994	1.32	0.0132	96.0 (15)	102 (14)	111 (2.6)	105 (11)	89.5 (9.9)	103 (14)
Benzyl Benzoate	0.9986	2.53	0.0253	0.9998	0.04	0.000400	113 (6.1)	103 (3.5)	93.3 (3.3)	99.2 (6.0)	93.2 (1.3)	103 (4.3)
Benzyl Salicilate	0.9926	2.62	0.0262	0.9930	0.54	0.00540	114 (3.9)	102 (2.7)	114 (4.9)	114 (1.0)	103 (4.1)	104 (0.90)
Benzyl Cinnamate	0.9945	2.95	0.0295	0.9963	0.59	0.00590	114 (7.7)	112 (3.5)	105 (3.8)	102 (1.4)	107 (0.34)	101 (2.5)

[a] Equivalent to µg g^{-1}. [b] Calculated by GC-MS/MS. [c] Initial concentration (µg g^{-1}). Rinse-off: Limonene: 0.115; benzyl alcohol: 0.932; hexylcinnamal: 1.15; benzyl salicylate: 0.450. Leave-on: 0.273. --: not detected.

Table 5. Linearity and limits of detection of the method for the GC-MS and GC-MS/MS analysis of preservatives.

	GC-MS (SIM)						GC-MS/MS (SRM)					
	Non derivatized			Derivatized			Non derivatized			Derivatized		
Preservatives	Correlation coefficient (R)	IDL (ng mL^{-1})	LOD[a] (%, w/w × 10^4)	Correlation coefficient (R)	IDL (ng mL^{-1})	LOD[a] (%, w/w × 10^4)	Correlation coefficient (R)	IDL (ng mL^{-1})	LOD[a] (%, w/w × 10^4)	Correlation coefficient (R)	IDL (ng mL^{-1})	LOD[a] (%, w/w × 10^4)
Bronidox	0.9977	3.05	0.0305	0.9999	2.24	0.0224	0.9998	0.15	0.00150	0.9999	0.11	0.00110
Phenoxy Ethanol	0.9967	2.81	0.0281	0.9990	1.50	0.0150	0.9996	1.50	0.0150	0.9998	1.21	0.0121
Methyl paraben	0.9972	3.02	0.0302	0.9995	0.58	0.00580	0.9982	0.86	0.00860	1.0000	0.010	0.000100
BHA	0.9984	2.20	0.0220	0.9979	0.98	0.00980	0.9996	0.091	0.000900	0.9993	0.032	0.000320
BHT	0.9990	0.60	0.00600	0.9993	0.57	0.00170	0.9996	0.051	0.000500	1.0000	0.031	0.000310
Ethyl paraben	0.9974	3.00	0.0300	0.9996	0.53	0.00530	0.9978	0.91	0.00910	0.9999	0.059	0.00590
Isopropyl paraben	0.9972	2.90	0.0290	0.9997	0.48	0.00480	0.9989	0.58	0.00580	1.0000	0.018	0.000180
Propyl paraben	0.9956	2.92	0.0292	0.9998	0.46	0.00460	0.9980	0.95	0.00950	1.0000	0.098	0.00100
IPBC	0.9956	10	0.100	0.9984	8.20	0.0820	0.9978	3.73	0.0373	0.9986	2.80	0.0280
Isobutyl paraben	0.9941	2.84	0.0284	0.9995	0.44	0.00440	0.9972	0.58	0.00580	0.9999	0.042	0.000420
Butyl paraben	0.9942	2.92	0.0292	0.9998	0.63	0.00630	0.9979	0.75	0.00750	0.9999	0.098	0.00100
Triclosan	0.9915	5.71	0.0571	0.9997	0.20	0.00200	0.9911	2.04	0.0204	1.0000	0.089	0.000900
Benzyl paraben	0.9942	5.90	0.0590	0.9984	0.46	0.00460	0.9920	2.90	0.0290	0.9997	0.12	0.00120

[a] Equivalent to µg g^{-1}.

In general, GC-MS and GC-MS/MS showed similar linearity, repeatability and reproducibility. But with GC-MS/MS, lower IDLs and LODs (up to 1 order of magnitude) were obtained. In addition, the use of MS/MS transitions (SRM) instead of selected ions (SIM) improves selectivity which it represents a great advantage to detect and quantify trace levels of fragrance allergens and preservatives in real samples.

Derivatization step

All preservatives were satisfactory analyzed without the need of a derivatization step. Nevertheless, and with the objective of improving performance especially in terms of detection limits, the inclusion of a derivatization step was evaluated (see conditions in the experimental section). GC-MS and GC-MS/MS performance parameters for derivatized preservatives are summarized in **Table 5**. Correlation coefficients R≥0.998 were obtained in both cases. RSD values for GC-MS and GC-MS/MS analysis ranged from 0.05-10% for intra-day precision, and 0.73-15% for inter-day precision (**Table S3**).

Recovery values are shown in **Table 6 and S5,** being higher than 90% for the majority of the compounds with RSD values usually lower than 8%, similar to those obtained for the non derivatized preservatives. IDLs were calculated as the concentration giving a signal-to-noise ratio of three (S/N = 3) in all cases since none of the target compounds were detected in the solvent chromatographic blanks, excluding methyl paraben, propyl paraben and butyl paraben by GC-MS/MS analysis. In those

cases IDLs were estimated as the average amount of analyte giving a response that is the solvent chromatographic blanks plus three times the standard deviation. The obtained IDL values are also included in **Table 5**; as can be seen IDLs were up to 1 order of magnitude lower (parabens and triclosan) for both GC-MS and GC-MS/MS. Therefore, acetylation presents an advantage for the analysis of preservatives, because it permits improving the chromatographic peak shape as well as the chromatographic response, reducing detection limits.

Table 3. Recoveries of the method for the target preservatives. (RSD,%)[a].

	Non derivatized						Derivatized					
	Rinse-off[b]			Leave-on[c]			Rinse-off[b]			Leave-on[c]		
Preservatives	2 μg g^{-1}	10 μg g^{-1}	20 μg g^{-1}	2 μg g^{-1}	10 μg g^{-1}	20 μg g^{-1}	2 μg g^{-1}	10 μg g^{-1}	20 μg g^{-1}	2 μg g^{-1}	10 μg g^{-1}	20 μg g^{-1}
Bronidox	105 (10)	111 (12)	97.1 (2.5)	91.1 (12)	101 (9.9)	105 (1.8)	93.0 (10)	115 (13)	112 (5.3)	78.2 (4.5)	88.0 (0.83)	89.1 (10)
Phenoxy Ethanol	-	-	-	111 (3.7)	98.3 (5.7)	111 (2.2)	-	-	-	104 (15)	81 (3.8)	112 (15)
Methyl paraben	112 (1.7)	101 (1.7)	111 (5.3)	106 (6.6)	102 (12)	110 (2.3)	90.1 (7.6)	110 (9.5)	87.1 (5.6)	86.0 (12)	85.1 (5.2)	88.4 (3.8)
BHA	87.1 (5.5)	102 (5.1)	88.0 (5.4)	89.1 (11)	80.7 (2.9)	101 (2.3)	113 (14)	111 (4.8)	89.2 (12)	87.1 (12)	81.5 (1.7)	93.4 (3.3)
BHT	114 (3.0)	93.4 (2.5)	85.0 (2.9)	86.0 (10)	86.2 (1.4)	97.2 (1.5)	111 (12)	114 (5.6)	108 (10)	85.4 (5.9)	81 .4 (1.7)	99.5 (7.9)
Ethyl paraben	88.2 (6.0)	124 (11)	112 (15)	114 (5.2)	108 (8.5)	111 (2.5)	108 (10)	87.2 (15)	100 (5.2)	86.4 (7.6)	88.3 (2.1)	99.0 (6.6)
Isoprpyl paraben	103 (15)	85.0 (1.7)	109 (3.7)	111 (7.1)	96.3 (9.1)	108 (2.3)	111 (6.1)	113 (3.4)	107 (9.1)	87.3 (6.2)	88.0 (1.6)	103 (7.8)
Propyl paraben	112 (8.5)	89.0 (3.1)	112 (5.5)	114 (4.7)	98.1 (9.1)	109 (2.1)	94.0 (3.2)	112 (1.2)	105 (3.7)	86.5 (12)	89.7 (2.0)	103 (5.7)
IPBC	97.2 (12)	86.3 (9.1)	106 (9.8)	107 (6.3)	113 (5.9)	106 (6.0)	93.4 (11)	114 (15)	111 (3.4)	113 (11)	96.1 (9.2)	112 (4.8)
Isobutyl paraben	109 (6.0)	85.1 (1.6)	109 (5.9)	111 (8.3)	101 (6.3)	104 (2.3)	113 (6.0)	100 (2.4)	102 (4.2)	90.2 (6.4)	88.4 (1.7)	102 (4.5)
Butyl paraben	113 (2.1)	90.1 (4.2)	113 (4.8)	107 (6.9)	99.1 (12)	102 (3.0)	115 (4.3)	114 (4.2)	106 (4.0)	93.3 (7.9)	89.6 (2.7)	105 (4.5)
Triclosan	110 (2.1)	88.1 (7.8)	86.3 (12)	110 (6.7)	90.0 (2.4)	113 (5.3)	114 (6.2)	113 (5.7)	107 (0.89)	97.0 (4.1)	90.3 (5.2)	105 (2.6)
Benzyl paraben	85.0 (2.8)	89.3 (1.9)	86.2 (6.3)	114 (7.4)	104 (6.9)	113 (5.1)	114 (3.8)	111 (3.3)	113 (0.77)	103 (11)	104 (2.3)	107 (2.6)

[a] Calculated by GC-MS/MS. [b]Initial concentration (μg g^{-1}): Phenoxyethanol: 1838; isobutyl paraben: 1.30. [c] Initial concentration (μg g^{-1}): Methyl paraben: 0.247. --: Not calculated since initial sample concentration is higher than the spiked level.

3.4. Application to real samples

The validated method was applied to the analysis of 17 real cosmetic and personal care samples including 5 rinse-off (shampoos, toothpaste, shower gel and a liquid soap) as well as 12 leave-on (baby moisturizing lotion, body milks, sunblock, lipstick, gloss lipstick, deodorants, nail polish remover, regenerative cream) products, with the intention of demonstrating method adequacy for a wide variety of the most common cosmetic products. We have not included perfumes, colognes and eau the toilette, since these cosmetic formulations do not require any sample pretreatment other than dilution before GC analysis [36,37]. Results are shown in **Table 7**. The recoveries of benzyl alcohol-d_7 and MeP-d_4 (surrogate standards) were satisfactory, with values generally close to 90%.

The analysis of those groups of cosmetic ingredients or additives is complex due to the own nature of the cosmetic samples and due to the fact that the analytes can be present from traces concentrations at the sub μg g^{-1} level up to several thousands of μg g^{-1} (range of four orders of magnitude). The inclusion of injection blanks (after each sample analysis) and process blanks is mandatory.

Table 7. Analysis of rinse-off and leave-on samples[a] (% w/wx10^4)[b].

	Rinse-off samples					Leave-on samples											
Fragrances	**Sh1**	**Sh2**	**Too**	**SG**	**LS**	**BML**	**SB**	**LP**	**GL**	**Deo1**	**Deo2**	**Deo3**	**Deo4**	**NPR**	**BM1**	**BM2**	**BM3**
Limonene	0.098	0.093	30.3	88.0	0.260	65.3	0.465	0.717	0.080	200	396	204	1.12	104	9.20	0.092	2.76
Benzyl Alcohol		7.01			5.27		4.36	2.93	1.31			11.2	6.24		41.4	0.084	
Linalool	8.02		12.9	544		360	39.3	101		1019	2028	185	316	0.643	39.6		167
Methyl-2-octynoate		2.24															
Citronellol		50.1		20.2		6.60		1.45	1.00	18.3	278		262	20.9	18.6		
Citral						7.29		6.28			17.8	61.3		4.77			
Geraniol		3.47		111	0.280	39.7		2.83					22.9	57.7	27.4		
Cinnamal										4.81				0.106			
Hydroxycitronellal	3.08				0.226		0.427			1.55					1.73	0.877	
Anise Alcohol							0.288								0.155	43.6	
Cinnamyl Alcohol							0.698								8.34	0.204	
Eugenol			1.44				3.48			49.5	0.182	45.8	3.45	43.8	0.135		
Methyl Eugenol															0.075		
Coumarin	9.82	10.1		26.3		6.17	0.986			566	50.0	26.5	168		31.8	0.041	
α-isomethylionone					0.024					1.20	171		145		48.2		
Lilial	0.844				0.069	357	1.24			4.49	675	598	476			0.038	
Lyral	1.76	1.56		3.33											19.0		
Farnesol												2119	2800				
Hexylcinnamal	14.5	233			33.3	0.631	19.4			7.43	2627	1147	638		135		1.12
Benzyl Benzoate				2.21	0.121	1.88	0.515		807	0.685	1313			0.587	11.5	0.054	
Benzyl Salicylate		0.271		1.65		2.10	18.3			1.01	781	887	154		18.6	0.179	
Preservatives[d]	**Sh1**	**Sh2**	**Too**	**SG**	**LS**	**BML**	**SB**	**LP**	**GL**	**Deo1**	**Deo2**	**Deo3**	**Deo4**	**NPR**	**BM1**	**BM2**	**BM3**
Phenoxy Ethanol	24.6	1.32	0.425	9.85	0.915	30.1	2351	1.45	1557				0.292		2973	2125	2037
Methyl paraben	5.45	0.212		2.31		7.45	1782		589				1.05		2079	3.34	2929
BHA							3.27										
BHT				0.160	0.130	1057	14.5	2996	9.63	6.23	174	13.4	29.4				
Ethyl paraben				0.824		2.31	200		149						36.7		2267
Propyl paraben	0.986					2.76	635	1494	283						884	0.474	2574
IPBC													17.8				
Isobutyl paraben	0.888						94.9									0.508	
Butyl paraben	1.70					0.662	177									1.10	
Triclosan			0.370							6.66	5763	3271					
Benzyl alcohol_d_7[c]	119	104	94	106	83	91	114	113	91	96	95	108	101	99	101	105	115
Methyl paraben_d_4[c]	95	82	91	84	105	86	96	78	83	95	83	79	89	99	94	102	115

Sh: shampoo, Too: toothpaste; SG: shower gel; LS: liquid soap; BML: baby moisturizing lotion, SB: sunblock, LP: lipstick, GL: gloss lipstick, Deo: deodorant, NPR: nail polish remover, BM: body milk. [b]Equivalent to μg g^{-1}.[c]Surrogate recovery, (%). [d]Parabens concentration expressed as acid (% w/w ×10^4).

Fragrance allergens

All analyzed samples contained fragrance allergens. Limonene, linalool and coumarine were found in most of the samples reaching concentration values up to 0.2% (2000 μg g^{-1}). Also lilial, hexylcinnamal, citronellol, benzyl benzoate and benzyl salicylate were found in many samples, with concentrations between 0.04 and 3000 μg g^{-1}. Other fragrance allergens were found in 2-9 samples. Among them, the presence of farnesol in two samples at high levels of concentration (2119 and 2800 μg g^{-1}) must be highlighted, while methyl-2-octynoate and methyl eugenol were found in a shampoo and a body milk at low levels (<2.3 μg g^{-1}). Regarding the number of compounds by cosmetic sample, a body milk (BM1) sample contained sixteen of target fragrance allergens, while in the other cosmetic samples, the number of these compounds was between 3-12, highlighting a deodorant (Deo2) with high concentrations (> 0.1%) of linalool, hexylcinnamal and benzyl benzoate. EU Regulation limits, established for benzyl alcohol, hydroxycitronellal, methyleugenol and isoeugenol, were fulfilled in all cases. Nevertheless, labeling requirements (see introduction) were not complied in 25% of the samples.

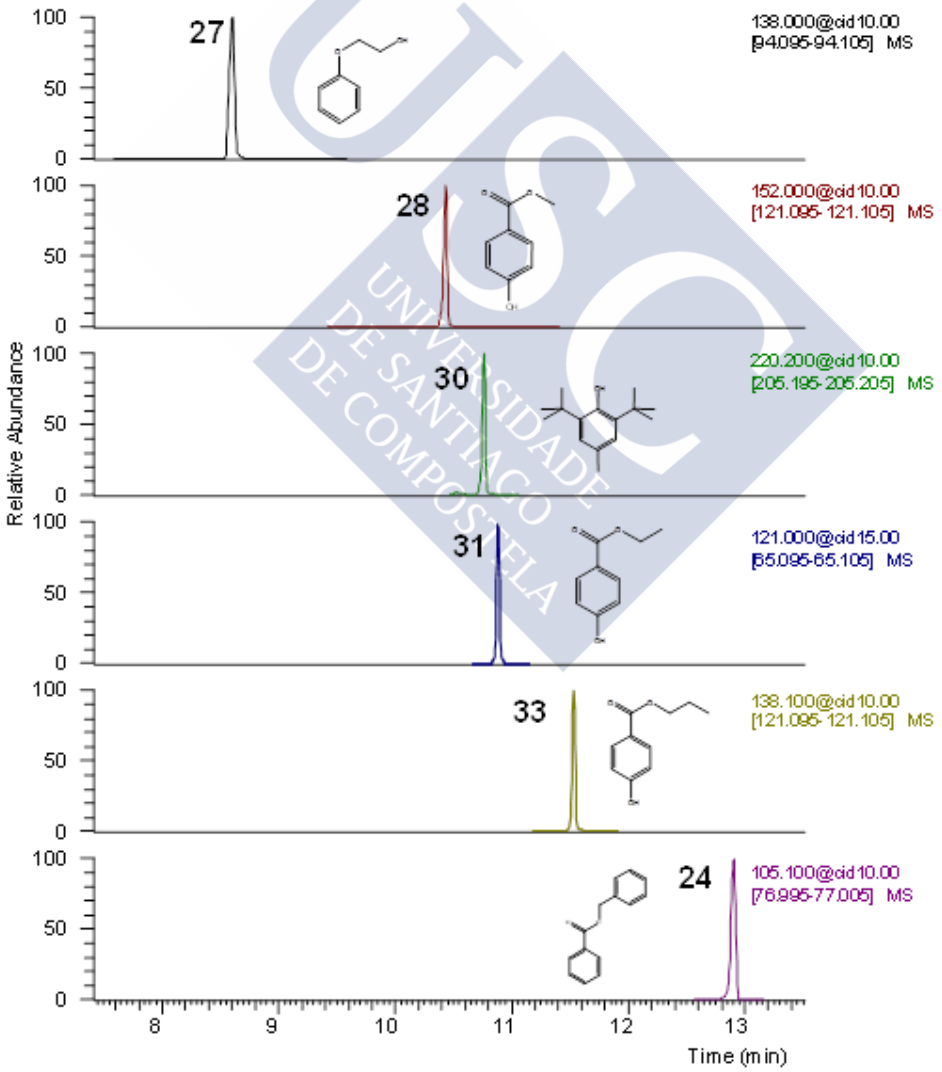

Fig. 4. GC-MS/MS transition chromatogram for a gloss lipstick (GL) (see compound key in **Table 1** and sample concentration in **Table 7**)

Preservatives

In the case of preservatives, they were present in all samples being phenoxyethanol the most frequently found (76% of the samples) at very high concentration in four leave-on samples (>1500 μg g^{-1}, 0.15%), including a gloss lipstick (GL), although these levels do not exceed the maximum concentration permitted by European legislation (1%). **Figure 4** shows the GC-MS/MS transition chromatograms for this last sample. BHT was detected in eight samples, in some of them at high levels (baby care product BML, 1057 μg g^{-1}, lipstick LP, 0.3 %). This substance does not present any restriction of use in cosmetics. Five of the 7 studied parabens were found in the samples. The most common were methyl paraben (59%) with high levels (>1700 μg g^{-1}) in three leave-on samples (SB, BM1,BM3) and propyl paraben (47%). Other parabens, ethyl, butyl, and isobutyl, were found in 6, 4 and 3 cosmetics, respectively. The total paraben concentration was very high in one leave-on sample (BM3), close to the legal limit of 0.8%. Triclosan was detected in 4 samples, at very high concentration in two deodorants, reaching the limit established by the European regulation (0.3%) [3]. BHA and IPBC were detected at low concentration levels in one sample. The maximum number of target preservatives, eight, was found in a sunblock sample (SB), containing very high concentrations of phenoxyethanol (2350 μg g^{-1}), and methylparaben (1780 μg g^{-1}). Other cosmetic and personal care samples contained between 2-6 of the target preservatives. Trace levels of preservatives at the low and sub μg g^{-1} have been detected. Their presence may be caused by their use during manufacture of other cosmetics or as an impurity of other cosmetic ingredients, since those very low concentrations do not have antimicrobial effect.

Although regulation is not clear regarding preservatives labeling requirements, in general, parabens were declared when their concentration exceed 0.01%. Phenoxyethanol was included in the product label for four of the samples (concentrations above 0.1%) and triclosan appeared in the label of the two products containing the highest levels.

4. CONCLUSIONS

A micro-MSPD method followed by gas chromatography–mass spectrometry/gas chromatography-triple quadrupole-mass spectrometry (GC-MS/MS) has been optimized for the determination of two groups of cosmetic additives, fragrance allergens and preservatives. This study included a total of thirty eight target substances subjected to restrictions or requirements according to the EU Cosmetic Directive. Multivariate optimization by means of experimental design was carried out in real non-spiked rinse-off and leave-on personal care products. The micro-MSPD procedure involved the use of only 0.1 g of sample as well as very low amounts of sorbents and since it was performed in Pasteur glass pipettes it could be implemented in any laboratory at very low cost. The method followed by GC-MS / GC-MS/MS analyses was extensively validated in real samples showing satisfactory performance in terms of linearity, sensitivity, accuracy and precision with mean recoveries of 90 % and RSD values generally below 10%. The use of GC coupled to triple quadrupole mass detection (MS/MS) instead of single quadrupole (MS), enabled reaching very low detection limits (low ng g^{-1}). The inclusion of a derivatization step allowed improving the chromatographic peak shape as well as the chromatographic response for the phenolic preservatives. The validated method was applied to real cosmetic samples, with the aim of demonstrating the method suitability for a

wide variety of the most common personal care products. Most of the target substances were found in the samples at concentration levels from the sub parts per million to the parts per mill. Several fragrances and preservatives have been detected at levels above 0.1% (1000 μg $g^{-1)}$. Regarding compliance with the EU regulation, maximum concentration limits were fulfill although two deodorants and a baby care product presented concentration of triclosan (0.3 %) and parabens (0.8%) at the legal limits, respectively. In addition, several samples did not fulfill the labeling requirements for fragrance allergens.

Acknowledgements

This research was supported by FEDER funds and projects CTQ2010-19831 (Ministerio de Ciencia e Innovación, Spain) and CN 2012/299 (Consolidated Research Groups Program, Xunta de Galicia).

Appendi A. Supplemetary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2014.03.070.

References

[1] European Commission. Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. Opinion concerning fragrance allergy in consumers, 1999. Available at http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/index_en.htm (accessed December 2013).

[2] U. Klaschka, Int. J. Hyg. Environ. Health, 213 (2010) 308.

[3] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast), in Off. J. Eur. Union L342/59, (2009). Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF(accessed January 2014).

[4] A. Chisvert, A. Salvador, in A.Salvador, A. Chisvert (Eds), Analysis of Cosmetic Products, Elsevier, Amsterdam, 2009, pp. 978.

[5] Twenty-sixth Commission Directive 2002/34/EC of 15 April 2002 adapting to technical progress Annexes I, II and VII to Council Directive 76/768/ECC on the approximation of the laws of the Member States relating to cosmetic products. Available at: http://eur-lex.europa. eu/LexUriServ /LexUriServ.do?uri=OJ:L:2002:102:0019:0031:EN:PDF (accesed January 2014).

[6] A. Chisvert, A. Salvador, in A. Salvador, A. Chisvert (Eds), Analysis of Cosmetic Products, Elsevier, Amsterdam, 2007, pp. 211.

[7] A. Andersen, Int. Toxicol., 27 (2008) 1.

[8] M.G. Soni, I.G. Carabin, G.A. Burdock, Food. Chem. Toxicol., 43 (2005) 985.

[9] P.D. Darbre, P.W. Harvey, J. Appl. Toxicol., 28 (2008) 561.

[10] G.J. Nohynek, C.J. Borgert, D. Dietrich, K.K. Rozman, Toxicol. Lett., 223 (2013) 295.

[11] DHI Water and Environment. Environmental Study of Enhancing the Endocrine Disruptor Priority List with a focus of low production volume chemicals. Revised Report to DG Environ. Hersholm, Denmark, 2007. Available at: http://ec.europa.eu/environment/endocrine/documents/final_report_2007.pdf (accessed January 2014).

[12] M. Pedrouzo, F. Borrull, R.M. Marce, E. Pocurull, Tr. Anal. Chem., 30 (2011) 749.

[13] T.P. Tsiallou, V.A. Sakkas, T.A. Albanis, J. Sep. Sci., 35 (2012) 1659.

[14] E. Becerril-Bravo, J.P. Lamas, L. Sanchez-Prado, M. Lores, C. Garcia-Jares, B. Jimenez, M. Llompart, Chemosphere, 81 (2010) 1378.

[15] E. Becerril, J.P. Lamas, L. Sanchez-Prado, M. Llompart, M. Lores, C. Garcia-Jares, Talanta, 83 (2010) 464.

[16] J.P. Lamas, L. Sanchez-Prado, C. Garcia-Jares, M. Llompart, J. Chromatogr. A, 1217 (2010) 1882.

[17] J.P. Lamas, L. Sanchez-Prado, M. Lores, C. Garcia-Jares, M. Llompart, J. Chromatogr. A, 1217 (2010) 5307.

[18] I. Masuck, C. Hutzler, A. Luch, J. Sep. Sci., 34 (2010) 2686.

[19] I. Masuck, C. Hutzler, A. Luch, J. Chromatogr. A, 1217 (2010) 3136.

[20] J.P. Lamas, L. Sanchez-Prado, C. Garcia-Jares, M. Lores, M. Llompart, J. Chromatogr. A, 1217 (2010) 8087.

[21] C. Devos, N. Ochiai, K. Sasamoto, P. Sandra, F. David, J. Chromatogr. A, 1255 (2012) 207.

[22] M. Pedrouzo, F. Borrull, R.M. Marce, E. Pocurull, J. Chromatogr. A, 1216 (2009) 6994.

[23] J. Regueiro, E. Becerril, C. Garcia-Jares, M. Llompart, J. Chromatogr. A, 1216 (2009) 4693.

[24] M. Diaz-Alvarez, E. Turiel, A. Martin-Esteban, Int. J. Environ. Anal. Chem., 93 (2013) 727.

[25] F. Vela-Soria, O. Ballesteros, I. Rodriguez, A. Zafra-Gomez, L. Ballesteros, R. Cela, A. Navalon, Anal. Bioanal. Chem., 405 (2010) 7259.

[26] P.G. Wang, W. Zhou, J. Sep. Sci., 36 (2013) 1781.

[27] L. Sanchez-Prado, J.P. Lamas, M. Lores, C. Garcia-Jares, M. Llompart, Anal. Chem., 82 (2010) 9384.

[28] G. Alvarez-Rivera, T. Dagnac, M. Lores, C. Garcia-Jares, L. Sanchez-Prado, J.P. Lamas, M. Llompart, J. Chromatogr. A, 1270 (2012) 41.

[29] L. Sanchez-Prado, G. Alvarez-Rivera, J.P. Lamas, M. Llompart, M. Lores, C. Garcia-Jares, Anal. Methods, 5 (2013) 416.

[30] L. Sanchez-Prado, G. Alvarez-Rivera, J.P. Lamas, M. Lores, C. Garcia-Jares, M. Llompart, Anal. Bioanal. Chem., 401 (2011) 3293.

[31] M. Llompart, M. Celeiro, J. Pablo Lamas, L. Sanchez-Prado, M. Lores, C. Garcia-Jares, J. Chromatogr. A, 1293 (2013) 10.

[32] International Fragrance Association (IFRA), 2003. Analytical procedure. Part 1, J. Agric. Food Chem. 51 (2003) 6398. Available at: http://www.ifraorg.org/view_document.asp?docid= 22183 (accessed February 2014)

[33] A. Chaintreau, E. Cicchetti, N. David, A. Earls, P. Gimeno, B. Grimaud, D. Joulain, N.Kupfermann, G. Kuropka, F. Saltron, C. Schippa, J. Chromatogr. A, 1218 (2011) 7869.

[34] L. Qving, Z. Qing, L. Wentao, L. Haiyu, L. Pi, M. Qiang, M. Xianshuang, Q. Meiling, B. Hua, J. Sep. Sci., 36 (2013) 3534.

[35] P.G Wang, W. Zhou, W.G Wamer, A.J. Krynitsky, J.I. Radre, Anal. Methods, 4 (2012) 3612.

[36] L. Sanchez-Prado, M. Llompart, J.P. Lamas, C. Garcia-Jares, M. Lores, Talanta, 85 (2011) 370.

[37] M. Lopez-Nogueroles, J.L. Benede, A. Chisvert, A. Salvador, Anal. Methods 5 (2013) 409.

Apendix A. Supplementary data

Table S1. Retention time, selected MS ions (SIM) and transitions (SRM) for fragrance allergens

Compound	GC-MS (SIM)		GC -MS/MS (SRM)	
Fragrance allergens	Retention time	**Quantification** and identification ions[a]	Retention time	Precursor ion → Product ion (Colision energy, eV)
Limonene	6.65	**68**, 93,121	6.08	67.9 → 53.0 (10) **92.8 → 77.0 (15)** 92.8 → 91.0 (10)
Benzyl Alcohol	6.72	77, 79, **108**	6.13	79.0 → 77.0 (10) 108.2 → 77.0 (25) **108.2 → 79.1 (15)**
Linalool	7.61	**71** ,93 ,121	7.14	71.0 → 43.0 (10) **92.9 → 77.0 (10)** 92.9 → 91.0 (10)
Methyl-2-octynoate	8.74	79 ,**95** ,123	8.34	79.0 → 77.0 (10) 94.9 → 55.1 (10) **94.9 → 67.0 (10)**
Citronellol	9.02	**69** ,95 ,109	8.63	**67.1 → 41.0 (15)** 68.9 → 39.0 (15) 68.9 → 41.1 (10)
Citral	9.16/9.41	**69,** 94, 109	8.76/9.03	39.0 → 38.2 (15) 68.9 → 39.1 (15) **93.9 → 79.0 (10)**
Geraniol	9.25	**69**, 93 ,111	8.87	68.9 → 39.0 (15) 68.9 → 41.0 (10) **92.9 → 77.1 (10)**
Cinnamal	9.51	77, 103, **131**	9.11	103.1 → 77.0 (10) 131.1 → 51.0 (40) **131.1 → 77.0 (25)**
Hydroxycitronellal	9.55	**59**, 71 , 81	9.18	**59.0 → 31.0 (10)** 59.0 → 43.0 (25) 71.0 → 43.0 (10)
Anise Alcohol	9.57	109, 121, **138**	9.19	**109.1 → 77.0 (15)** 109.1 → 94.0 (10) 137.0 → 77.0 (20)
Cinnamyl Alcohol	9.77	**92**, 105, 115	9.39	**92.1 → 91.0 (10)** 134.1 → 78.0 (15) 134.1 → 91.2 (20)
Eugenol	10.10	103,131,**164**	9.75	131.1 → 103.0 (10) 164.2 → 103.0 (20) **164.2 → 149.1 (10)**
Methyleugenol	10.39	147, 163, **178**	10.05	178.1 → 77.0 (35) **178.1 → 147.1 (10)** 178.1 → 163.1 (10)
Isoeugenol	10.75	103, 131, **164**	10.12/10.41	77.0 → 50.9 (15) 103.0 → 77.0 (10) **164.1 → 149.1 (10)**
Coumarin	10.76	90, 118, **146**	10.40	118.0 → 89.0 (20) 118.0 → 90.1 (10) **146.1 → 118.1 (10)**
α-isomethyl ionone	10.93	107, **135**, 150	10.59	107.0 → 91.0 (10) **150.1 → 91.0 (20)** 150.1 → 135.1 (10)

Table S1. Continuation

Lilial	11.28	131, 147, **189**	10.92	**189.2 → 131.1 (10)** 204.2 → 147.2 (10) 204.2 → 189.2 (10)
Amyl Cinnamal	12.18	115, **129**, 145	11.75	116.8 → 115.1 (10) **128.9 → 128.0 (20)** 202.0 → 129.1 (10)
Lyral	12.35	79, 93, **136**	11.91	92.9 → 77.0 (15) 92.9 → 77.0 (10) **136.1 → 79.0 (10)**
Amylcinnamyl Alcohol	12.47	91, 115, **133**	12.04	91.1 → 65.0 (15) **133.1 → 55.0 (10)** 133.1 → 115.1 (10)
Farnesol	12.47/12.70	**69**, 93, 107	12.06/12.27	69.0 → 39.0 (15) 69.0 → 41.0 (10) **108.9 → 67.0 (10)**
Hexylcinnamal	13.08	**129**, 145, 216	12.59	116.8 → 91.0 (15) **129.0 → 127.0 (20)** 216.3 → 129.1 (10)
Benzyl Benzoate	13.41	77, 91, **105**	12.86	90.9 → 65.0 (15) **105.1 → 77.0 (10)** 194.1 → 165.1 (20)
Benzyl Salicilate	14.78	65, **91**, 228	14.09	91.0 → 39.0 (30) 91.0 → 65.0 (15) **228.1 → 91.1 (10)**
Benzyl Cinnamate	18.43	**91**, 131, 192	17.74	131.1 → 77.0 (20) 131.1 → 103.0 (10) **192.0 → 191.2 (15)**

Table S2. Retention time, selected ions (SIM) and transitions (SRM) for preservatives.

	GC-MS (SIM)				GC-MS/MS (SRM)			
	Non derivatized		Derivatized		Non derivatized		Derivatized	
Preservatives	Retention Time	**Quantification** and identification ions	Retention Time	**Quantification** and identification ions	Retention Time	Precursor ion → Product ion (Colision Energy, eV)	Retention time	Precursor ion → Product ion (Colision Energy, eV)
Bronidox	8.95	85.0, 106.9, **136.9**	8.95	85.0, 106.9, **136.9**	8.52	134.9 → 107.0 (5) **137.0 → 109.0 (5)**	6.59	**134.9 → 107.0 (5)** 137.0 → 109.0 (5)
Phenoxyethanol	8.99	77.0, **94.0**, 138.0	10.28	77.0, **87.0**, 94.0	8.58	94.0 → 61.1 (15) 94.0 → 65.8 (10) **138.0 → 94.1 (5)**	8.30	87.0 → 85.1 (5) **93.9 → 65.7 (10)**
Methyl paraben	10.74	93.0, **121.0**, 152.0	10.92	93.0, **121.0**, 152.0	10.41	121.0 → 65.1 (15) 121.0 → 93.1 (10) **152.0 → 121.1 (10)**	9.22	121.0 → 65.1 (15) 121.0 → 93.1 (10) **152.0 → 121.1 (10)**
BHA	10.96	137.0, **165.1**, 180	11.51	137.0, **165.1**, 180.1	10.63	137.1 → 77.1 (20) **165.2 → 137.1 (10)** 180.1 → 165.1 (10)	9.30	137.1 → 77.1 (20) **165.2 → 137.1 (10)** 180.1 → 165.1 (10)
BHT	11.10	177.0, **205.1**, 220.2	11.10	177.0, **205.1**, 220.2	10.76	205.1 → 145.1 (15) 205.1 → 177.2 (10) **220.2 → 205.2 (10)**	9.48	205.1 → 145.1 (15) 205.1 → 177.2 (10) **220.2 → 205.2 (10)**
Ethyl paraben	11.19	**121.0**, 138.0, 166.0	11.41	**121.0**, 138.0, 166.0	10.85	**121.0 → 65.1 (15)** 121.0 → 93.1 (10) 138.0 → 121.0 (10)	9.88	121.0 → 65.1 (15) 121.0 → 93.1 (10) **138.0 → 121.0 (10)**
Isopropyl paraben	11.39	**121.0**, 138.0, 180.1	11.63	**121.0**, 138.0, 180.1	11.04	121.0 → 65.1 (15) 121.0 → 93.0 (10) **138.1 → 121.1 (10)**	10.16	121.0 → 65.1 (15) 121.0 → 93.0 (10) **138.1 → 121.1 (10)**
Propyl paraben	11.90	**121.0**, 138.0, 180.0	12.18	**121.0**, 138.0, 180.0	11.52	121.0 → 65.1 (15) 121.0 → 93.0 (10) **138.1 → 121.1 (10)**	10.75	121.0 → 65.0 (15) 121.0 → 93.1 (10) **138.1 → 121.0 (10)**
IPBC	12.28	100.0, **164.9**, 181.9	12.28	100.0, **164.9**, 181.9	11.85	164.9 → 126.9 (35) **181.9 → 153.9 (10)**	10.88	164.9 → 126.9 (35) **181.9 → 153.9 (10)**
Isobutyl paraben	12.39	93.0, **121.0**, 138.0	12.69	93.0, 121.0, **138.0**	11.96	121.1 → 65.1 (15) 121.1 → 93.1 (10) **138.0 → 121.1 (10)**	11.22	121.1 → 65.1 (15) 121.1 → 93.1 (10) **138.0 → 121.1 (10)**
Butyl paraben	12.80	**121.0**, 138.0, 194.1	13.16	121.0, **138.0**, 194.1	12.34	121.1 → 65.1 (15) 121.1 → 93.1 (10) **138.0 → 121.1 (10)**	11.59	121.1 → 65.1 (15) 121.1 → 93.1 (10) **138.0 → 121.1 (10)**
Triclosan	18.53	218.0, **287.9**, 289.9	19.20	218.0, **287.9**, 289.9	17.84	**218.0 → 126.9 (30)** 218.0 → 155.1 (20) 288.0 → 218.0 (15)	14.62	218.0 → 126.9 (30) 218.0 → 155.1 (20) **288.0 → 218.0 (15)**
Benzyl paraben	18.84	91.0, **121.0**, 228.0	19.16	91.0, **121.0**, 228.0	18.26	121.0 → 65.1 (20) **121.0 → 93.1 (10)** 228.2 → 121.1 (10)	14.58	121.0 → 65.1 (20) 121.0 → 93.1 (10) **228.2 → 121.1 (10)**

Table S3. Intra-day and inter-day method precision. (RSD, %)

	GC-MS (SIM)				GC-MS/MS (SRM)			
	Intra-day precision		Inter-day precision		Intra-day precision		Inter-day precision	
Fragrances	50 ng mL^{-1}	500 ng mL^{-1}	50 ng mL^{-1}	500 ng mL^{-1}	50 ng mL^{-1}	500 ng mL^{-1}	50 ng mL^{-1}	500 ng mL^{-1}
Limonene	1.2	1.8	3.4	3.1	1.3	1.2	3.7	7.2
Benzyl Alcohol	0.30	2.5	4.1	1.3	4.9	1.8	9.8	6.2
Linalool	0.39	2.8	4.1	2.5	1.6	1.3	3.4	6.4
Methyl-2-octynoate	1.9	1.9	10	3.2	2.5	0.75	4.5	6.1
Citronellol	4.6	2.3	7.7	5.6	3.4	0.96	7.3	9.5
Citral	6.2	3.0	9.1	3.6	3.7	0.98	6.4	5.8
Geraniol	2.7	5.0	9.6	8.4	1.8	0.71	11	7.4
Cinnamal	0.90	1.8	8.5	3.4	2.8	1.9	2.8	2.8
Hydroxycitronellal	2.0	2.6	7.1	3.3	3.7	1.1	5.7	7.8
Anise Alcohol	3.2	2.5	7.8	1.0	3.1	1.8	5.3	3.7
Cinnamyl Alcohol	5.5	2.2	11	0.96	1.9	1.9	4.8	5.1
Eugenol	7.1	2.3	5.2	0.61	2.4	2.1	10	4.8
Methyleugenol	1.2	1.4	3.0	1.8	3.6	1.1	4.1	5.6
Isoeugenol	7.3	2.0	4.6	0.63	5.2	3.6	7.7	7.7
Coumarin	5.4	1.3	6.7	2.6	2.2	3.6	5.2	3.9
α-isomethyl ionone	0.83	1.9	6.7	2.7	1.9	0.56	1.8	3.7
Lilial	0.46	2.0	5.4	2.1	4.8	1.3	5.5	4.2
Amyl Cinnamal	1.6	1.9	8.9	4.1	1.7	1.4	4.3	2.5
Lyral	3.4	2.8	8.5	3.1	4.9	1.7	5.9	6.6
Amylcinnamyl Alcohol	8.3	2.1	12	1.2	4.5	3.1	9.7	13
Farnesol	-	0.5	-	9.2	7.0	6.1	12	10
Hexylcinnamal	3.2	1.9	8.2	4.4	3.8	1.8	2.9	2.8
Benzyl Benzoate	0.24	1.4	6.2	2.8	4.0	1.5	6.6	3.0
Benzyl Salicylate	1.3	2.1	8.2	1.6	5.7	7.1	9.8	10
Benzyl Cinnamate	3.2	1.7	8.4	3.5	5.9	5.7	5.5	8.5

	Intra-day precision				Inter-day precision				Intra-day precision				Inter-day precision			
	50 ng mL^{-1}		500 ng mL^{-1}		50 ng mL^{-1}		500ng mL^{-1}		50 ng mL^{-1}		500 ng mL^{-1}		50ng mL^{-1}		500 ng mL^{-1}	
Preservatives[a]	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D
Bronidox	2.8	5.9	1.9	0.67	3.8	8.0	1.7	3.7	2.2	0.84	1.8	1.7	6.6	6.5	1.7	3.6
Phenoxy Ethanol	0.23	1.8	2.4	0.56	6.2	7.7	3.1	4.7	2.6	3.8	1.6	1.1	3.6	6.5	4.5	1.4
Methyl paraben	0.16	3.8	1.7	4.4	6.3	10	0.73	8.4	6.9	1.0	4.8	2.3	9.1	5.3	7.4	5.3
BHA	0.05	5.1	2.0	2.6	4.1	6.5	1.3	4.8	1.9	1.5	1.1	3.4	7.7	3.7	4.8	4.9
BHT	5.5	6.2	1.4	3.2	2.9	8.2	1.4	3.7	1.9	1.4	1.1	1.2	3.6	4.3	5.4	7.7
Ethyl paraben	1.6	3.2	2.0	0.46	5.5	8.2	0.73	5.4	5.2	1.5	5.0	2.4	7.8	5.4	4.9	4.4
Isopropyl paraben	0.92	3.9	1.7	1.8	5.6	5.5	0.81	8.6	4.3	0.91	4.3	2.1	9.7	5.1	6.0	4.5
Propyl paraben	1.2	2.0	2.0	0.19	7.9	5.7	1.1	5.0	7.1	1.3	6.3	1.1	5.6	2.1	7.1	8.3
IPBC	0.91	5.2	1.7	7.5	5.9	3.0	2.9	6.5	9.9	4.1	5.9	6.3	8.1	7.7	4.4	5.0
Isobutyl paraben	0.74	2.6	1.6	0.40	6.4	5.6	1.0	4.8	5.4	1.2	6.0	1.6	5.3	6.9	8.5	7.8
Butyl paraben	0.86	1.6	1.8	0.60	8.9	6.7	2.0	4.5	5.6	1.1	7.3	1.5	5.8	6.4	8.9	4.6
Triclosan	1.3	0.7	2.2	1.2	13	3.0	12	1.9	2.1	0.45	5.9	3.5	8.9	4.3	13	3.9
Benzyl paraben	1.9	1.3	3.1	1.9	7.9	5.3	11	2.6	15	1.6	10	1.8	15	5.2	15	3.1

[a] ND: Non derivatized; D: Derivatized

Table S4. Recoveries for fragrance allergens in cosmetics and personal care products (%, RSD)[a]

	BM2		LS			SG	Sh	SB	BM3	LP
Fragrances	**2 µg g^{-1}**	**20 µg g^{-1}**	**2 µg g^{-1}**	**10 µg g^{-1}**	**20 µg g^{-1}**	**10 µg g^{-1}**	**10 µg g^{-1}**	**10 µg g^{-1}**	**10 µg g^{-1}**	**10 µg g^{-1}**
Limonene	71.2 (7.2)	78.6 (6.7)	69.1 (7.5)	75.2 (7.2)	72.0 (12)	-	90.1 (1.7)	77.2 (2.0)	93.1 (0.87)	96.2 (4.2)
Benzyl Alcohol	114 (2.8)	92.5 (5.7)	-	98.1 (1.9)	107 (5.6)	115 (5.0)	109 (0.19)	91.2 (10)	116 (0.38)	113 (12)
Linalool	97 (1.4)	96.3 (3.9)	81.4 (5.8)	80.2 (2.3)	95.3 (9.5)	-	117 (0.28)	-	-	-
Methyl-2-octynoate	100 (5.0)	94.8 (5.2)	93.5 (5.7)	84.3 (0.69)	97.2(9.2)	102 (5.7)	93.1 (3.2)	81.2 (9.7)	113 (6.1)	96.2 (6.3)
Citronellol	102 (1.8)	108 (4.0)	91.8 (5.0)	85.5 (2.3)	109 (9.8)	-	105 (6.7)	100 (2.2)	100 (2.8)	98.1 (12)
Citral	112 (4.3)	108 (4.5)	112 (7.3)	83.4 (5.1)	102 (4.6)	113 (5.3)	116 (7.2)	94.5 (4.0)	108 (8.1)	107 (13)
Geraniol	103 (6.9)	97.0 (4.7)	108 (8.8)	88.0 (0.98)	114 (0.27)	-	93.4 (1.9)	92.3 (4.1)	97.8 (7.3)	111 (14)
Cinnamal	105 (1.9)	101 (5.9)	97.6 (4.4)	82.8 (1.5)	101 (3.6)	95.0 (7.8)	103 (2.8)	97.8 (4.6)	108 (2.5)	100 (9.5)
Hydroxycitronellal	111 (2.5)	93.2 (4.7)	89.1 (10)	72.4 (2.8)	95.0 (3.9)	93.1 (5.2)	102 (6.8)	81.2 (7.4)	111 (6.8)	93.1 (9.1)
Anise Alcohol	-	-	99.1 (9.5)	89.3 (4.2)	111 (1.6)	98.3 (7.9)	101 (4.2)	96.3 (6.1)	104 (0.041)	96.0 (12)
Cinnamyl Alcohol	110 (6.2)	101 (7.9)	100 (13)	89.2 (4.2)	103 (1.3)	98.9 (8.7)	97.2 (6.7)	90.9 (8.8)	109 (3.5)	83.5 (12)
Eugenol	87.1 (3.5)	101 (10)	88.2 (4.6)	90.2 (2.4)	100 (1.6)	96.5 (7.8)	95.3 (4.2)	92.3 (3.6)	109 (5.9)	100 (1.2)
Methyleugenol	88.1 (2.5)	95.0 (5.6)	99.1 (5.7)	88.7 (1.6)	98.2 (3.5)	92.1 (5.7)	110 (0.45)	96.0 (4.9)	106 (3.7)	93.0 (8.1)
Isoeugenol	91.3 (14)	89.1 (11)	90.0 (1.5)	85.2 (3.3)	98.1 (3.8)	100 (9.2)	93.5 (2.9)	82.4 (5.3)	95.2 (0.45)	113 (3.2)
Coumarin	101 (2.1)	103 (6.5)	96.1 (1.3)	85 .2 (0.74)	96.4 (2.5)	-	118 (0.62)	81.4 (1.5)	97.3 (0.52)	108 (7.1)
α-isomethyl ionone	85.1 (2.6)	98.3 (5.4)	91.2 (4.0)	86.2 (1.0)	105 (4.3)	86.4 (5.9)	104 (1.9)	93.9 (0.24)	103 (2.5)	108 (8.1)
Lilial	91.6 (3.3)	97.4 (6.1)	86.8 (4.6)	78.2 (1.4)	99.3 (1.6)	90.0 (5.3)	98.1 (1.2)	85.2 (0.10)	102 (4.2)	93.5 (9.0)
Amyl Cinnamal	98.7 (3.4)	100 (6.5)	87.4 (6.9)	87.2 (3.5)	99.4 (2.8)	101 (9.0)	107 (0.82)	96.2 (3.6)	106 (4.2)	114 (13)
Lyral	108 (3.1)	102 (6.7)	104 (2.6)	76.3 (5.1)	98.0 (2.6)	76.1 (7.3)	98.2 (1.5)	82.5 (11)	114 (7.5)	114 (12)
Amylcinnamyl Alcohol	109 (13)	105 (6.7)	90.9 (8.8)	98.1 (4.2)	109 (3.1)	85.5 (9.9)	98.2 (6.7)	96.2 (9.4)	111 (9.1)	113 (13)
Farnesol	n.d	94.3 (5.1)	n.d	89.0 (4.9)	98.3 (4.7)	112 (15)	95 (0.70)	96.0 (13)	106 (12)	89.2 (15)
Hexylcinnamal	93.3 (3.3)	97.4 (6.4)	-	-	-	94.4 (8.7)	-	-	112 (5.9)	100 (5.8)
Benzyl Benzoate	93.8 (1.0)	91.2 (6.3)	87.3 (4.6)	85.1 (2.1)	100 (3.5)	95.8 (8.7)	105 (0.43)	95.5 (1.2)	113 (5.1)	97.2 (7.4)
Benzyl Salicylate	110 (3.7)	80.4 (7.3)	100 (13)	109 (3.7)	102 (2.9)	115 (10)	115 (6.9)	-	114 (4.3)	94.3 (15)
Benzyl Cinnamate	109 (3.7)	91.5 (7.5)	103 (5.5)	115 (7.5)	113 (3.2)	107 (12)	116 (0.14)	112 (0.40)	115 (4.8)	108 (12)

BM: Body milk, LS: liquid soap, SG: shower gel, Sh: shampoo, SB: sunblock, LP: lipstick. [a.] See initial concentrations in **Table 7**. n.d: Not detected

Table S5. Recoveries for preservatives in cosmetics and personal care products (%, RSD).

	BM2				LS						SG	Sh	SB	BM3	LP
Preservatives	2 μg g^{-1}	2 μg g^{-1b}	20 μg g^{-1}	20 μg g^{-1b}	2 μg g^{-1}	2 μg g^{-1b}	10 μg g^{-1}	10 μg g^{-1b}	20 μg g^{-1}	20 μg g^{-1b}	10 μg g^{-1}	10 μg g^{-1}	10 μg g^{-1}	10 μg g^{-1}	10 μg g^{-1}
Bronidox	95.1 (1.2)	96.2 (2.9)	100 (6.6)	113 (6.2)	91.1 (4.3)	107 (9.5)	91.1 (1.4)	83.1 (2.6)	101 (5.9)	96.0 (8.6)	89.1 (7.0)	101 (2.8)	108 (0.90)	95.1 (5.2)	86.0 (4.1)
Phenoxy Ethanol	-	-	-	-	90.2 (10)	88.9 (7.6)	93.0 (1.5)	80.4 (4.9)	101 (4.0)	100 (9.9)	85.0 (5.0)	-	-	-	101 (4.7)
Methyl paraben	-	-	107(6.5)	110 (4.9)	110 (4.3)	103 (9.7)	90.0 (6.3)	89.5 (5.5)	98.0 (0.74)	100 (1.13)	109 (7.6)	100 (7.7)	-	-	110 (5.9)
BHA	88.3 (0.70)	106 (1.1)	95.7 (8.4)	103 (1.1)	85.1 (8.3)	107 (5.7)	85.1 (1.1)	93.1 (3.0)	100 (0.96)	93.4 (7.7)	95.4 (7.3)	94.1 (0.89)	83.2 (0.91)	102 (2.7)	108 (3.6)
BHT	75.2 (4.5)	86.3 (10)	91.0 (7.7)	102 (8.9)	75.2 (5.2)	90.9 (0.22)	77.4 (0.70)	80.4 (3.6)	101 (3.5)	105 (9.2)	114 (15)	92.1 (0.10)	-	100 (3.2)	-
Ethyl paraben	104 (5.6)	97.1 (2.9)	108 (6.3)	109 (7.8)	97.3 (8.6)	95.6 (3.1)	89.0 (5.6)	87.2 (4.9)	98.3 (0.33)	95.3 (13)	102 (1.0)	92.2 (9.8)	-	-	91.5 (5.4)
Isopropyl paraben	108 (8.3)	105 (2.6)	105 (3.1)	113 (11)	106 (8.9)	85.5 (13)	94.2 (4.3)	94.2 (0.10)	97.2 (2.0)	100 (12)	106 (1.0)	114 (8.5)	115 (1.2)	103 (3.8)	106 (6.5)
Propyl paraben	110 (6.6)	91.4 (13)	104 (9.2)	108 (10)	87.1 (3.6)	104 (9.3)	100 (5.6)	89.1 (6.3)	101 (1.4)	1004(1.1)	102 (1.1)	115 (1.2)	-	-	-
IPBC	91.2 (10)	92.1 (4.8)	100 (7.7)	108 (8.0)	97.0 (4.3)	92.8 (6.3)	94.0 (12)	94.7 (0.43)	97.9 (1.3)	100 (12)	96.0 (0.42)	84.3 (10)	113 (15)	114 (10)	105 (7.1)
Isobutyl paraben	109 (8.9)	93.9 (8.8)	105 (7.2)	107 (8.7)	92.5 (12)	115 (6.8)	98.3 (5.3)	96.9 (4.8)	102 (2.5)	1004(8.7)	85.2 (10)	111 (5.3)	-	106 (1.2)	103 (2.9)
Butyl paraben	107 (1.5)	99.1 (1.4)	101 (9.3)	107 (6.9)	104 (12)	107 (8.1)	104 (6.1)	91.9 (3.4)	106 (4.8)	103 (9.7)	114 (15)	107 (0.79)	-	103 (3.8)	106 (5.3)
Triclosan	115 (4.6)	103 (6.9)	111 (11)	100 (11)	103 (13)	103 (11)	95.2 (2.5)	95.2 (3.5)	115 (6.7)	92.5 (5.1)	86.4 (4.2)	96.2 (3.0)	106 (13)	110 (3.5)	113 (1.3)
Benzyl paraben	107 (7.8)	106 (10)	94.5 (11)	104 (9.1)	114 (15)	111 (9.9)	109 (13)	109 (1.7)	109 (6.8)	92.9 (15)	87.5 (5.1)	108 (7.9)	116 (9.0)	102 (3.5)	113 (4.3)

BM: body milk, LS: liquid soap, SG: shower gel, Sh: shampoo, SB: sunblock, LP: lipstick. [a] See initial concentrations in **Table 7**.

[b] Derivatized. Blank cells: Not calculated since initial sample concentration is higher than the spiked

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1.3. IN-VIAL MICRO-MATRIX-SOLID PHASE DISPERSION FOR THE ANALYSIS OF FRAGRANCE ALLERGENS, PRESERVATIVES, PLASTICIZERS, AND MUSKS IN COSMETICS

Cosmetics (Ed. MDPI, Switzerland)
ISSN: 2079-9284

Cosmetics, 1 (2014) 171-201. **doi: 10.3390/cosmetics1030171**

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IN-VIAL MICRO-MATRIX-SOLID PHASE DISPERSION FOR THE ANALYSIS OF FRAGRANCE ALLERGENS, PRESERVATIVES, PLASTICIZERS, AND MUSKS IN COSMETICS

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ABSTRACT

Fragrance allergens, preservatives, plasticizers, and synthetic musks are usually present in cosmetic and personal care products formulations and many of them are subjected to use restrictions or labeling requirements. Matrix-solid-phase-dispersion (MSPD) is a very suitable analytical technique for the extraction of these compounds providing a simple, low cost sample preparation, and the possibility of performing both extraction and clean-up in one step, reducing possible contamination and analyte losses. This extraction technique has been successfully applied to many cosmetics ingredients allowing obtaining quantitative recoveries. A new very simple micro-MSPD procedure performing the disruption step in a vial is proposed for the gas chromatography-mass spectrometry (GC-MS) analysis of 66 chemicals usually present in cosmetics and personal care products. The method was validated showing general recoveries between 80% and 110%, relative standard deviation (RSD) values lower than 15%, and limits of detection (LODs) below 30 $ng \cdot g^{-1}$. The validated method was applied to a broad range of cosmetics and personal care products, including several products intended for baby care.

Keywords: cosmetics; micro-matrix-solid-phase-dispersion; fragrance allergens; preservatives; plasticizers; musks; gas chromatography-mass spectrometry (GC-MS)

1. INTRODUCTION

Fragrances and preservatives are common ingredients in cosmetics and personal care products. Fragrances provide nice and attractive scents and preservatives are used to prevent microbial growth because the aqueous nature of many personal care products is an optimal medium for microbial growth. European legislation [1] requires the monitoring of 26 volatile compounds, the so-called potentially allergen substances (PAS) or fragrance allergens. Their presence must be indicated in the list of ingredients when their concentrations exceed 0.01% for rinse-off products, and 0.001% for leave-on products. Of these 26 substances, 24 are chemically defined volatile compounds whereas the other two are natural moss extracts. One of these 24 fragrance allergens, lyral®, was recently proposed to be transferred to the Annex III (list of substances which cosmetic products must not contain except subject to restrictions) to Annex II (list of substances prohibited in cosmetic products). Also, pinene and methyleugenol were included in the referred study; pinene is proposed to be labelled when its concentration exceeds 0.01% for rinse-off products, and 0.001% for leave-on products, whereas methyleugenol has been banned in cosmetics and personal care products for some years, and now it is included in Annex III.

Parabens are the most frequently used preservatives (their maximum concentration in cosmetics and personal care products are 0.4% for a single ester and 0.8% for mixture of esters). Its extended use is due to their broad antimicrobial spectrum and low cost [2,3]. Although these compounds are not mutagenic agents, recent studies have reported that certain parabens have been associated with genotoxicity, allergies and may also act as antiandrogens [4,6]. In recent years, another preservative, phenoxyethanol, is increasing its use as substitute hof parabens. According to the European regulation [1] the maximum concentration permitted for this compound is 1% regardless of its use. However, a recent study reported by the France National Agency for Security of Medicaments (ANSM) proposed not using phenoxyethanol in products intended for children under 3 years and to reduce the maximum permitted concentration (0.4%) in other personal care products [7]. Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) and the bromine-containing preservative bronidox, are also preservatives present in personal care products. Their maximum permitted concentrations according European legislation is 0.3% and 0.1%, respectively. IPBC (iodopropynyl butylcarbamate) is not permitted in products for children under 3 years of age, except in bath products, shower gels and shampoo. The antioxidants butylated hidroxyanisole (BHA) and butylated hydroxytoluene (BHT) can be used without restrictions.

Synthetic musks are other chemical compounds usually present in personal care products under the term "fragrance" or "parfum". Synthetic musks are used as an alternative for natural musks. The European regulation has forbidden the use of three nitromusks: musk ambrette, musk moskene and musk tibetene due to their bioaccumulative properties [8]. Another two nitromusks (musk ketone and musk xylene) are allowed with restrictions[10][1].

Plasticizers (phthalates and adipates) are used in cosmetic and personal care formulations as solvents, fixer of fragrances, and to promote skin penetration. Diethyl phthalate (DEP) can be present in personal care products as solvent of the synthetic musk galaxolide. However, the European Commission on Endocrine Disruption has listed DEP as a Category 1 priority substance [9]. Other six phthalates (dibutyl phthalate (DBP), dimethoxyethyl phthalate (DMEP), diisopentylphthalate (DIIP), dipentyl phthalate (DPP), benzylbutyl phthalate (BBP) and di(2-ethylhexyl) phthalate (DEHP)) were forbidden as ingredients in cosmetics and personal care products

due to their possible carcinogenic and mutagenic effects in human health. Adipates (1,6-dimethylhexanedioate (DMA), 1,6-diethylhexanedioate (DEA) and di(2-ethylhexyl) adipate (DEHA)) are permitted without restrictions.

In order to guarantee product safety, the development of analytical methods is mandatory in cosmetic quality control. In this way, several analytical methods to determine fragrance allergens, preservatives, plasticizers, and/or musks in cosmetics and personal care products have been reported. A summary of the more recent extraction and analysis techniques for the analysis of these compounds in different cosmetic matrices can be found in recent reviews [10-13].

Matrix-solid-phase-dispersion (MSPD) is a very suitable analytical technique for the extraction of contaminants in environmental and other matrices [1] as well as to determine fragrances, preservatives, plasticizers and musks in cosmetic samples. This technique is primarily used because of its flexibility and selectivity providing efficient and low cost extractions; the possibility of performing extraction and clean-up in one step is one of their main advantages [15-21]. Also, its miniaturizing allows reducing the amount of sample, reagents and solvents required. MSPD combines different aspects of several analytical techniques, performing sample disruption while dispersing the components of the sample on and into a solid support, thereby generating a chromatographic material that possesses a particular character for the extraction of compounds from the dispersed sample [2]. This extraction technique allowed obtaining quantitative recoveries for many cosmetic ingredients [16,19,20,22].

For very volatile compounds such as pinene and limonene, that are easily lost during extraction processes [23], MSPD can constitute a good alternative to lower analyte losses [20].

The aim of the present study is to compare the performance of two micro-MSPD procedures, performing the sample disruption in mortar and also in vial, for the gas chromatography-mass spectrometry (GC-MS) analysis of 66 compounds including fragrance allergens, preservatives, plasticizers, and musks, usually present in cosmetics and personal care products. All these families of compounds are subjected to restrictions according international regulation.

2. EXPERIMENTAL SECTION

2.1. Chemicals, Materials and Samples

The analyzed compounds, their chemical names, CAS numbers, suppliers, purity of the standards and European legislation restrictions are also shown in Table 1. Deuterated methyl-4-hydroxybenzoate-2,3,5,6-d_4 (MeP_d_4; 98atom% D), benzyl_d_7 alcohol (98atom% D) and di(2-ethylhexyl)phthalate-3,4,5,6-d_4 (DEHP_d4; 98atom% D) used as surrogate standard, were obtained from C/D/N Isotopes (Quebec, Canada), Aldrich (St. Louis, MO, USA), and Fluka Chemie GmbH (Steinheim, Germany), respectively. 2,4,6-trichlorobiphenyl (PCB-30) used as internal standard was provided by Dr. Ehrenstorfer (Augsburg, Germany).

Ethyl acetate was provided by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Florisil (60–100 mesh) was purchased from Supelco Analytical (Bellefonte, PA, USA) and anhydrous sodium sulphate (99%) from Panreac (Barcelona, Spain).

Individual stock solutions were prepared in acetone, isooctane or methanol. Further dilutions and mixtures were prepared in acetone or ethyl acetate. Solutions were stored in amber glass vials at −20°C. All solvents and reagents were of analytical grade.

Metallic, glass, and ceramic materials; sorbents (Florisil and sodium sulphate anhydrous) and the glass wool for laboratory use (Sigma-Aldrich) were baked at 230 °C for 12 h before use to eliminate possible phthalate contamination. All materials were allowed to cool down wrapped with aluminum foil and Florisil and sodium sulphate anhydrous in desiccator.

Samples of cosmetics and personal care products from national and international brands were obtained from local sources. They included leave-on and rinse-off products such as shampoo, shower gel, body milk, sunblock, among others, including products intended for babies. Until their analysis, samples were kept in their original containers at room temperature.

Table 1. Target compounds: chemical names, suppliers, purity, CAS and European restrictions.

Fragrance Allergens	Chemical Names	Purity (%)	CAS	Maximum Concentration Permitted [1]
Pinene	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl	≥99 [b]	80-56-8	n.r
Limonene [a]	(4R)-1-Methyl-4-(1-methylethenyl)cyclohexene	97 [b]	5989-27-5	n.r
Benzyl alcohol [a]	Benzene methanol	≥99 [b]	100-51-6	1% (as preservative)
Linalool [a]	3,7-Dimethyl-1,6-octadien-3-ol	97 [b]	78-70-6	n.r
Methyl-2-octynoate [a]	Methyl heptin carbonate	≥99 [b]	111-12-6	n.r
Citronellol [a]	(±)-3,7-Dimethyoct-6-en-1-ol	95 [b]	106-22-9	n.r
Citral [a]	3,7-Dimethyl-2,6-octadienal	95 [b]	5392-40-5	n.r
Geraniol [a]	3,7-Dimethyl-(2E)-2,6-octadien-1-ol	≥96 [b]	106-24-1	n.r
Cinnamal [a]	3-Phenyl-2-propenal	≥93 [b]	104-55-2	n.r
Hydroxycitronellal [a]	7-Hydroxy-3,7-dimethyloctanal	≥95 [b]	107-75-5	1%
Anise alcohol [a]	4-Methoxybenzyl alcohol	98 [b]	105-13-5	n.r
Cinnamyl alcohol [a]	3-Phenyl-2-propen-1-ol	98 [b]	104-54-1	n.r
Eugenol [a]	2-Methoxy-4-(2-propenyl)-phenol	99 [b]	97-53-0	n.r
Methyleugenol [a]	1,2-Dimethoxy-4-(2-propenyl)-benzene	99 [b]	93-15-2	0.01% (fine fragrance); 0.004% (eau de toilette); 0.002% (fragrance cream); 0.0002% (other leave-on products); 0.001% (rinse-off products)
Isoeugenol [a]	2-Methoxy-4-(1-propenyl)phenol	98 [b]	97-54-1	0.02%
Coumarin [a]	2H-1-benzopyran-2-one	≥99 [b]	91-64-5	n.r
α-isomethyl ionone [a]	3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1yl)-3-buten-2-one	≥85 [b]	127-51-5	n.r
Lilial® [a]	2-(4-*tert*-Butylbenzyl)propionaldehyde	≥90 [b]	80-54-6	n.r
Amyl cinnamal [a]	2-Benzylideneheptanal	97 [b]	122-40-7	n.r
Lyral® [a,g]	Hydroxyhexyl-3-cyclohexene carboxaldehyde	≥97 [b]	31906-04-4	n.r
Amylcinnamyl alcohol [a]	2-Pentyl-3-phenylprop-2-en-1-ol	≥85 [b]	101-85-9	n.r
Farnesol [a]	3,7,11-trimethyldodeca-2,6,10-trien-1-ol	95 [b]	4602-84-0	n.r
Hexylcinnamal [a]	2-Benzylideneoctanal	≥95 [b]	101-86-0	n.r

Table 1. *Cont.*

Fragrance Allergens	Chemical Names	Purity (%)	CAS	Maximum Concentration Permitted [1]
Benzyl benzoate [a]	Phenylmethyl benzoate	≥99 [b]	120-51-4	n.r
Benzyl salicylate [a]	Benzyl-2-hydroxybenzoate	≥99 [b]	118-58-1	n.r
Benzyl cinnamate [a]	3-Phenyl-2-propenoic acid phenylmethyl ester	99 [b]	103-41-3	n.r
Preservatives				
Bronidox	5-Bromo-5-nitro-1,3-dioxane	≥99 [c]	30007-47-7	0.1% (rinse-off products)
Phenoxyethanol (phEtOH)	2-Phenoxyethanol	99 [c]	122-99-6	1%
Methyl paraben (MeP)	Methyl 4-hydroxibenzoate	99 [b]	99-76-3	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
BHA	Butylated hidroxyanisole	98.5 [c]	25013-16-5	n.r
BHT	Butylated hydroxytoluene	99 [c]	128-37-0	n.r
Ethyl paraben (EtP)	Ethyl 4-hydroxybenzoate	99 [b]	120-47-8	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
Isopropyl paraben (iPrP) *	Isopropyl 4-hydroxybenzoate	≥99 [b]	4191-73-5	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
Propyl paraben (PrP)	Propyl 4-hydroxybenzoate	99 [b]	94-13-3	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
IPBC	Carbamic acid, butyl-3-iodo-2-propynyl ester	97 [c]	55406-53-6	Prohibited in products for children under 3 years, except in bath products. Prohibited in oral and lip products. 0.02% (rinse-off products); 0.01% (leave-on products); 0.0075% (deodorants).
Isobutyl paraben (iBuP) *	Isobutyl 4-hydroxybenzoate	≥97 [b]	4247-02-3	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
Butyl paraben (BuP)	Butyl 4-hydroxybenzoate	99 [b]	94-26-8	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)

Table 1. *Cont.*

Preservatives	Chemical Names	Purity (%)	CAS	Maximum Concentration Permitted [1]
Triclosan	2,4,4′-Trichloro-2′-hydroxydiphenyl ether	≥97 [c]	3380-34-5	0.3% (toothpastes, hand soaps, shower gels, deodorants, face powders and blemish concealers, nail products); 0.2% (mouthwashes)
Benzyl paraben (BzP) *	Benzyl hydroxybenzoate	99 [b]	94-18-8	0.4% as acid (for single ester) 0.8% as acid (for mixtures of esters)
Plasticizers				
DMA	1,6-Dimethylhexanedioate	99 [c]	627-93-0	n.r
DEA	1,6-Diethylhexanedioate	99 [c]	141-28-6	n.r
DMP	Dimethyl phthalate	98 [c]	131-11-3	n.r
DEP	Diethyl phthalate	98 [b]	84-66-2	n.r
DIBP	Diisobutyl phthalate	99 [f]	84-69-5	n.r
DBP	Dibutyl phthalate	99 [b]	84-74-2	Prohibited
DMEP	Dimethoxyethyl phthalate	94 [f]	117-82-8	Prohibited
DPP	Dipentyl phthalate	99.2 [b]	131-18-0	Prohibited
BBP	Benzylbutyl phthalate	98 [b]	85-68-7	Prohibited
DEHA	Di(2-ethylhexyl) adipate	98.5 [c]	103-23-1	n.r
DIHP	Diisoheptylphthalate	99 [b]	41451-28-9	n.r
DEHP	Di(2-ethylhexyl) phthalate	99.5 [c]	117-81-7	Prohibited
DCHP	Diclohexyl phthalate	99 [b]	84-61-7	n.r
DPhP	Diphenyl phthalate	98 [b]	84-62-8	n.r
DNOP	Di-noctyl phthalate	≥ 98 [d]	117-84-0	n.r
Musks				
Cashmeran	1,1,2,3,3-Pentamethyl-2,5,6,7-tetrahydroinden-4-one	≥ 95 [f]	33704-61-9	n.r
Celestolide	4-Acetyl-6-tert-butyl-1,1-dimethylindane	≥ 98 [f]	13171-00-1	n.r
Phantolide	6-Acetyl-1,1,2,3,3,5-hexamethylindan	≥ 98 [f]	15323-35-0	2% (leave-on products)

Table 1. *Cont.*

Musks	Chemical Names	Purity (%)	CAS	Maximum Concentration Permitted [1]
Musk Ambrette	6-tert-Butyl-3-methyl-2,4-dinitroanisole	99 [f]	83-66-9	Prohibited
Traseolide	5-Acetyl-3-isopropyl-1,1,2,6-tetramethylindane	99 [f]	68140-48-7	n.r
Galaxolide	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta(g)-2-benzopyran	55.5 [b]	1222-05-5	n.r
Musk Xylene	1-tert-Butyl-3,5-dimethyl-2,4,6-trinitrobenzene	100 ng·mL^{-1} [c]	81-15-2	Prohibited in oral products. 1.0% (fine fragrance); 0.4% (eau de toilette); 0.03% (other products)
Tonalide	6-Acetyl-1,1,2,4,4,7-hexamethyltetralin	98 [f]	1506-02-1	Prohibited in oral products. 0.2% (rinse-off products) 0.1% (leave-on products, except: 1% hydroalcoholic products; 2.5% fine fragrance; 0.5% fragrance cream)
Musk Moskene	1,1,3,3,5-Pentamethyl-4,6-dinitro-2H-indene	≥99 [f]	116-66-5	Prohibited
Musk Tibetene	1-tert-Butyl-3,4,5-trimethyl-2,6-dinitrobenzen	≥99 [f]	145-39-1	Prohibited
Ambrettolide	17-Oxacycloheptadec-6-en-1-one	≥ 97 [b]	7779-50-2	n.r
Musk Ketone	4-tert-Butyl-3,5-dinitro-2,6-dimethyl acetophenone	≥98 [b]	81-14-1	Prohibited in oral products. 1.4% (fine fragrance) 0.56% (eau de toilette) 0.042% (other products)

[a] The presence of the substance must be indicated in the list of ingredients when its concentration exceeds 0.001% (leave-on products) and 0.01% (rinse-off products); [b] Sigma Aldrich Chemie GmbH (Steimheim, Germany); [c] Fluka Chemie GmbH (Steimheim, Germany); [d] Supelco Analytical (Bellefonte, PA, USA); [e] LGC Standards GmbH (Wesel, Germany); [f] Dr. Ehrenstorfer (Ausburg, Germany); [g] Is proposed to be excluded completely from cosmetics and personal care products n.r: no restricted by EC No 1223/2009. * Banned from 30 July 2015.

2.2. Micro-Matrix-Solid-Phase-Dispersion (MSPD)

Cosmetic samples (0.1 g) were exactly weighted into a 10-mL glass vial and spiked with 25 µL of each surrogate solution (10 µg·mL^{-1}) containing benzyl alcohol-d_7, MeP-d_4, PrP-d_4 and DEHP-d_4. Then, the sample was gently blended with 0.2 g of a drying agent (anhydrous Na_2SO_4), and 0.4 g of the dispersing sorbent (Florisil), into the vial or in a porcelain mortar, using a glass rod or a porcelain pestle, respectively, until a homogeneous mixture was obtained (*ca*. 5 min). The mixture was transferred into a glass Pasteur pipette (approximately 150 mm), with a small amount of glass wool at the bottom, containing 0.1 g of Florisil (to obtain a further degree of fractionation and sample clean-up). Finally, a small amount of glass wool was placed on top of the sample before compression with a spatula. Elution with ethyl acetate was made by gravity flow, collecting the extract into a 1 mL volumetric flask. Then, 12.5 µL of PCB-30 internal standard solution (1 µg·mL^{-1}) was added. The micro-MSPD extracts diluted when necessary were directly analyzed by GC-MS. Fortified samples were spiked with 20 µL of the corresponding acetone solution of the target compounds to get the desired final concentration and submitted to the same process described above. The optimization of the experimental conditions (amount of sample, solvent, dispersant and volume elution) has been described elsewhere [19,20]. **Figure 1** illustrates the described micro-MSPD process.

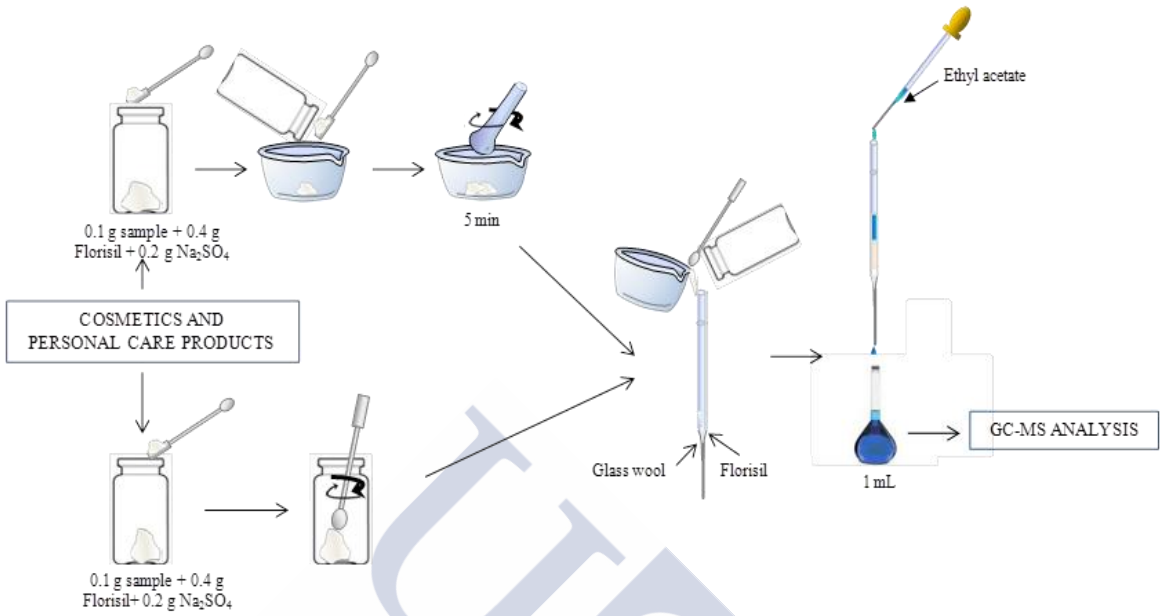

Figure 1. Micro-matrix-solid-phase-dispersion (MSPD) procedure.

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The analysis was performed using an Agilent 7890A (GC)-Agilent 5975C inert MSD with triple axis detector and an Agilent 7693 autosampler from Agilent Technologies (Palo Alto, CA, USA). The temperatures of the transfer line, the quadrupole and the ion source were set at 290, 150 and 230°C, respectively. Electronic impact (EI) was used as ionization technique. The system was operated by Agilent MSD ChemStation E.02.00.493 software.

Separation was performed on a ZB-5 capillary column (30 m × 0.25 mm i.d. (internal diameter, 0.25 µm film thickness) obtained from Phenomenex (Torrance, CA, USA). Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 mL·min^{-1}. The GC oven temperature was programmed from 60°C (held 1 min) to 100°C at 8°C min^{-1}, to 150°C at 20°C min^{-1}, to 200°C at 25°C min^{-1} to 220°C at 8°C min^{-1} and 30°C min^{-1} to 290°C (held 10 min). After 1 min, the split valve was opened (75 mL min^{-1}), and the injector temperature was kept at 260°C. The injection volume was 1 µL. The electron multiplier was set at a nominal value of 1553 V.

3. RESULTS AND DISCUSSION

3.1. GC-MS Performance

The chromatographic conditions were optimized to achieve an efficient separation of 66 target compounds frequently used in cosmetics and personal care products: 26 fragrance allergens, 13 preservatives, 15 plasticizers (phthalates and adipates) and 12 musks. For GC-MS analysis, the mass spectra detector (MSD) was operated in the selected ion monitoring (SIM) mode, monitoring three ions per compound. **Table 2** shows the quantification and identification ions, and the retention time of the compounds. Chromatograms of a standard solution containing 200 ng·mL^{-1} of target

compounds (DIHP, 400 ng mL^{-1}) are shown in **Figure 2**. The GC-MS method performance parameters for the 66 target compounds are summarized in **Table 3**. Regarding the instrumental linearity, the method exhibited a direct proportional relationship between the amount of each analyte and the chromatographic response. Calibration standards in ethyl acetate were prepared covering a concentration range from 10 to 1000 ng mL^{-1} (anise alcohol, cinnamyl alcohol, amylcinnamyl alcohol, triclosan, and musk ketone, 20–1000 ng mL^{-1}; IPBC, 50–1000 ng mL^{-1}; di-iso-heptyl-phthalate (DIHP), 100–4000 ng mL^{-1}; and farnesol, 250–1000 ng mL^{-1}). Correlation coefficients $R \geq 0.9915$ were generally obtained. Method precision was studied within-a-day (n = 3) and among-days (n = 6) at 250 ng mL^{-1} (other concentration levels, 50, 500 and 1000 ng mL^{-1} were calculated, data not shown). Relative standard deviation (RSD) values ranged from 1.7% to 9.5% for intra-day analysis, and between 1.8 and 10% for inter-day analysis. Instrumental detection limits (IDLs) were in all cases calculated as the concentration giving a signal-to-noise of three (S/N = 3) since none of the target compounds were detected in the solvent chromatographic blanks and they were at the low ng mL^{-1} with values in general below 6 ng mL^{-1} (farnesol, IPBC, and DIHP, 70 ng mL^{-1}, 10 ng mL^{-1}, and 24 ng mL^{-1}, respectively). The phthalate DIHP is complex mixtures of isomers, and the chromatographic signal is composed of several chromatographic peaks.

Table 2. Retention time, quantification and identification ions.

Key	Target Compounds	Retention Time (min)	Quantification and Identification Ions	Key	Target Compounds	Retention Time (min)	Quantification and Identification Ions
1	Pinene	5.23	77 (27), **93** (100), 121 (13)	34	Lyral®	12.45	79 (74), 93 (78), **136** (100)
2	Limonene	6.85	**68** (100), 93 (76),121 (25)	35	iBuP	12.49	93 (12), **121** (100), 138 (58)
3	Benzyl alcohol	6.90	77 (73), 79 (115), **108** (100)	36	Farnesol	12.63/12.93	**69** (100), 93 (27), 107 (15)
4	Linalool	7.77	**71 (100)** ,93 (84) ,121 (24)	37	Amylcinnamyl alcohol	12.64	91(88), 115 (60), **133** (100)
5	Methyl-2-octynoate	8.87	79 (66), **95** (100) ,123 (73)	38	BuP	12.93	**121** (100), 138 (84), 194 (6)
6	Bronidox	9.03	85 (27), 107 (49), **135** (100)	39	Celestolide	13.03	173 (22), **229** (100), 244 (44)
7	PhEtOH	9,09	77 (28), **94** (100), 138 (31)	40	Hexylcinnamal	13.31	**129** (100), 145 (51), 216 (40)
8	Citronellol	9.12	**69** (100) ,95 (49) ,109 (18)	41	Phantolide	13.53	187 (11), **229** (100), 244 (24)
9	DMA	9.23	101 (72), 111 (77), **114** (100)	42	Benzyl benzoate	13.58	77 (28), 91 (47), **105** (100)
10	Citral	9.27/9.51	**69** (100), 94 (17), 109 (10)	43	Ambrette	14.53	**253** (100), 254 (13), 268 (35)
11	Geraniol	9.36	**69** (100), 93 (18) ,111 (6)	44	Traseolide	14.74	43 (41), **215** (100), 258 (14)
12	Cinnamal	9.56	77 (35), 103 (50), **131** (100)	45	DIBP	14.84	57 (12), **149** (100), 223 (6.8)
13	Hydroxycitronellal	9.62	**59** (100), 71 (13) , 81 (43)	46	Galaxolide	14.84	213 (23), **243** (100), 258 (20)
14	Anise alcohol	9.64	109 (77), 121 (55), **138** (100)	47	Xylene	14.96	43 (62), 57 (16), **282** (100)
15	Cinnamyl alcohol	9.82	**92** (100), 105 (53), 115 (54)	48	Tonalide	14.99	43 (48), **243** (100), 258 (26)
16	Eugenol	10.21	103 (28),131 (27),**164** (100)	49	Benzyl salicylate	15.08	65 (11), **91** (100), 228 (12)
17	DEA	10.30	**111** (100), 128 (63), 157 (81)	50	Moskene	15.40	**263** (100), 264 (20), 278 (8.9)
18	Methyleugenol	10.47	147 (31), 163 (29), **178** (100)	51	Ambrettolide	16.23	**67** (100), 81 (98), 96 (89)
19	Isoeugenol	10.54/10.82	103(22), 131 (20), **164** (100)	52	Tibetene	16.33	43 (33), **251** (100), 266 (28)
20	MeP	10.78	93.0 (21), **121** (100), 153 (35)	53	DBP	16.46	**149** (100), 150 (9), 223 (4.9)
21	Coumarin	10.82	90 (42), 118 (110), **146** (100)	54	Ketone	16.99	191 (24), 294 (26), **279** (100)
22	DMP	10.83	77 (13), 194 (66), **163** (100)	55	DMEP	17.10	**59** (100), 104 (18), 149 (29)
23	BHA	11.03	137 (63), **165** (100), 180 (51)	56	Benzyl cinnamate	18.59	**91** (100), 131 (90), 192 (63)
24	α-isomethyl ionone	11.05	107 (58), **135** (100), 150 (61)	57	Triclosan	18.78	218 (93), **288** (100), 290 (93)
25	BHT	11.24	177 (8), **205** (100), 220 (25)	58	BzP	18.98	91 (46), **121** (100), 228 (21)
26	EtP	11.24	**121** (100), 138 (21), 166 (18)	59	DPP	19.12	71 (16), **149** (100), 237 (5.6)
27	Cashmeran	11.26	135 (43), **191** (100), 206 (57)	60	BBP	20.49	**91** (53), 149 (100), 206 (24)

Table 2. *Cont.*

Key	Target compounds	Retention time (min)	Quantification and identification ions	Key	Target compounds	Retention time (min)	Quantification and identification ions
28	Lilial®	11.36	131 (39), 147 (40), **189** (100)	61	DEHA	20.69	112 (26), **129** (100), 147 (21)
29	iPrP	11.45	**121** (100), 138 (39), 180 (14)	62	DIHP	21.07	149 (100), 223 (7), **265** (52)
30	DEP	11.82	**149** (100), 150 (12), 177 (24)	63	DCHP	21.53	55 (19), **149** (100), 167 (31)
31	PrP	11.99	**121** (100), 138 (58), 180 (7)	64	DEHP	21.54	167 (30), **149** (100), 279 (10)
32	Amyl cinnamal	12.31	115 (89), **129** (100), 145 (57)	65	DPhP	21.65	77 (19), 153 (4), **225** (100)
33	IPBC	12.34	100 (15), **165** (100), 182 (50)	66	DNOP	22.71	**149** (100), 223 (22), 279 (6.2)

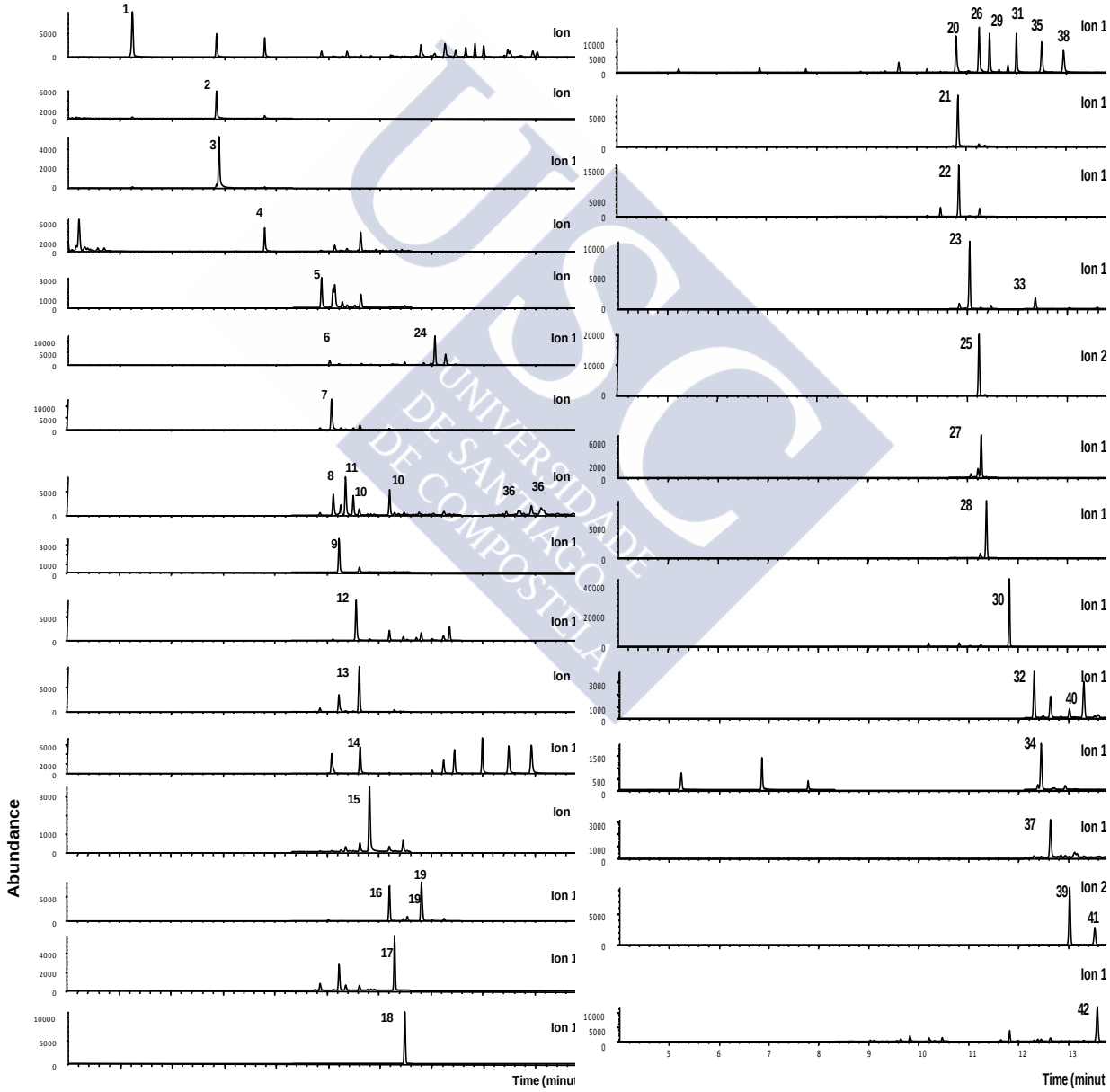

Figure 2. Selected ion monitoring (SIM) chromatogram of a standard mixture of the target compounds (200 ng·mL^{-1}; di-isoheptyl-phthalate (DIHP), 400 ng·mL^{-1}).

Table 3. Quality parameters of the method.

Fragrance Allergens	Correlation coefficient (*R*)	IDL ($ng \cdot mL^{-1}$)	LOD (%, w/w × 10^4)	LOQ (%, w/w × 10^4)	Intra-Day Precision (RSD, %) [a]	Inter-Day Precision (RSD, %) [b]
Pinene	0.9997	1.02	0.0169	0.0563	1.8	1.8
Limonene	0.9993	0.99	0.0213	0.0709	2.5	2.1
Benzyl alcohol	0.9980	2.03	0.0232	0.0773	3.0	5.5
Linalool	0.9984	2.10	0.0260	0.0866	4.0	3.8
Methyl-2-octynoate	0.9969	2.75	0.0275	0.0916	4.6	5.0
Citronellol	0.9965	2.78	0.0313	0.1042	5.6	4.6
Citral	0.9973	2.80	0.0400	0.1332	5.2	4.3
Geraniol	0.9969	3.05	0.0400	0.1332	5.1	5.2
Cinnamal	0.9984	2.97	0.0300	0.0990	3.4	3.2
Hydroxycitronellal	0.9972	1.93	0.0197	0.0656	4.7	4.2
Anise alcohol	0.9972	4.04	0.0404	0.1345	4.0	3.9
Cinnamyl alcohol	0.9965	5.28	0.0528	0.1758	3.3	3.8
Eugenol	0.9964	1.91	0.0210	0.0693	5.0	4.7
Methyleugenol	0.9982	1.97	0.0197	0.0656	2.8	2.4
Isoeugenol	0.9976	2.95	0.0309	0.1029	3.7	3.5
Coumarin	0.9997	2.00	0.0220	0.0733	1.7	2.1
α-isomethyl ionone	0.9985	0.96	0.0118	0.0393	3.4	3.0
Lilial®	0.9984	1.05	0.0196	0.0653	3.5	2.8
Amyl cinnamal	0.9963	2.20	0.0320	0.1066	4.4	4.1
Lyral®	0.9937	2.40	0.0240	0.0799	4.6	5.5
Amylcinnamyl alcohol	0.9930	6.04	0.0604	0.2011	4.3	4.1
Farnesol	0.9978	70.0	0.7000	2.331	4.3	2.3
Hexylcinnamal	0.9954	3.01	0.0301	0.1002	5.0	4.2
Benzyl benzoate	0.9986	2.10	0.0343	0.1142	3.1	2.7
Benzyl salicylate	0.9926	2.93	0.0293	0.0976	5.2	4.9
Benzyl cinnamate	0.9945	2.93	0.0293	0.0976	4.1	3.3

Table 3. *Cont.*

	Correlation coefficient (R)	IDL ($ng \cdot mL^{-1}$)	LOD (%, w/w × 10^4)	LOQ (%, w/w × 10^4)	Intra-Day Precision (RSD, %) [a]	Inter-Day (RSD, %) [b]	Precision
Preservatives							
Bronidox	0.9977	2.61	0.0261	0.0869	1.8	3.2	
PhEtOH	0.9967	2.34	0.0234	0.0779	4.6	4.4	
MeP	0.9972	2.02	0.0300	0.0999	4.2	5.3	
BHA	0.9984	1.7	0.0170	0.0566	4.5	4.8	
BHT	0.9990	0.53	0.0053	0.0176	3.1	2.6	
EtP	0.9974	2.87	0.0375	0.1249	3.3	3.7	
iPrP	0.9972	2.80	0.0380	0.1265	4.7	4.4	
PrP	0.9956	2.92	0.0292	0.0972	5.2	5.3	
IPBC	0.9956	10.0	0.150	0.4995	1.7	4.4	
iBuP	0.9941	2.94	0.0310	0.1032	5.1	5.2	
BuP	0.9942	3.02	0.0302	0.1006	4.4	5.4	
Triclosan	0.9915	5.95	0.0595	0.1981	4.8	5.7	
BzP	0.9942	5.90	0.0590	0.1947	5.0	7.9	
Plasticizers							
DMA	0.9994	0.90	0.0090	0.0299	1.8	2.1	
DEA	0.9992	1.20	0.0260	0.0866	3.6	3.0	
DMP	0.9996	0.47	0.0096	0.0319	2.5	2.1	
DEP	0.9996	0.70	0.0070	0.0233	3.0	3.0	
DIBP	0.9992	1.30	0.0203	0.0676	4.2	3.6	
DBP	0.9990	0.75	0.0075	0.0250	4.4	3.9	
DMEP	0.9991	2.00	0.0375	0.1238	4.3	4.3	
DPP	0.9982	0.17	0.0064	0.0213	4.6	4.8	
BBP	0.9976	2.00	0.0342	0.1139	2.5	3.6	
DEHA	0.9974	0.93	0.0261	0.0869	3.0	6.7	
DIHP	0.9989	24	0.4000	1.332	9.5	10	

Table 3. *Cont.*

Plasticizers	Correlation coefficient (R)	IDL ($ng \cdot mL^{-1}$)	LOD (%, w/w × 10^4)	LOQ (%, w/w × 10^4)	Intra-Day Precision (RSD, %) [a]	Inter-Day (RSD, %) [b]	Precision
DEHP	0.9976	0.95	0.0300	0.0999	3.9	6.3	
DCHP	0.9990	0.70	0.0200	0.0666	6.3	5.4	
DPhP	0.9990	0.45	0.0307	0.1022	3.6	5.2	
DNOP	0.9966	0.40	0.0092	0.0306	1.7	3.0	
Musks							
Cashmeran	0.9996	0.60	0.0300	0.0999	3.7	3.0	
Celestolide	0.9983	0.25	0.0026	0.0866	5.0	4.2	
Phantolide	0.9983	0.52	0.0087	0.0289	4.5	4.3	
Ambrette	0.9965	2.00	0.0300	0.0999	3.6	4.4	
Traseolide	0.9970	0.80	0.0126	0.0419	5.0	4.8	
Galaxolide	0.9995	0.83	0.0216	0.0719	3.6	2.8	
Xylene	0.9946	2.05	0.0293	0.0976	4.1	4.3	
Tonalide	0.9992	0.83	0.0162	0.0539	4.7	3.9	
Moskene	0.9933	1.72	0.0480	0.1598	2.6	4.3	
Tibetene	0.9964	1.90	0.0196	0.0652	4.2	4.0	
Ambrettolide	0.9990	2.13	0.1200	0.3996	3.8	3.5	
Ketone	0.9954	3.20	0.0706	0.2351	5.4	4.5	

[a] $n = 3$. Calculated for 250 $ng \cdot mL^{-1}$; [b] $n = 6$. Calculated for 250 $ng \cdot mL^{-1}$

3.2. Analytical Method Performance

Complete method quality parameters were evaluated using real cosmetic samples and the results are shown in **Tables 4 and 5**. In this way, recovery studies were carried out by applying the optimized method to two samples spiked at three levels of concentration: 2, 10 and 20 $\mu g\ g^{-1}$. These samples are a regenerating cream (leave-on) and a shampoo (rinse-off); they were selected for recoveries studies since the leave-on sample was labeled as perfume-free and preservative-free, and the rinse-off sample was almost free of the target compounds (only contained MeP, BHT, and PrP). In any case, previous analyses of the samples showed the presence of some of the target compounds, and these initial concentrations were taken into account to calculate the recoveries. Recoveries were higher than 90% for the most of the studied compounds (see **Tables 4 and 5** for leave-on and rinse-off samples, respectively) regardless of using vial or mortar for the MSPD disruption step. In the case of the most volatile compounds, pinene recovery was 70% and 35%, for

leave-on and rinse-of samples, respectively; and for limonene, recovery presented an average value of 75% employing a vial, whereas lower recoveries were obtained employing a mortar.

Recovery study was extended to three other cosmetic matrices (shampoo, sunblock product, body milk) that were fortified at 10 μg g^{-1}. Results are presented in **Table 6**, and demonstrate the quantitative recovery of the compounds. Precision was evaluated attaining RSD values generally lower than 10% (see also **Tables 4 and 5**).

Figure 3 shows a comparison of the results obtained using vial or mortar for the micro-MSPD for a real leave-on sample containing 23 target analytes (hands cream). Obtained responses are equivalent employing mortar or vial for the disruption step, excluding pinene and limonene for which responses were higher using vial. Limits of detection (LODs) were calculated as the compound concentration giving a signal-to-noise ratio of three (S/N = 3). As shown in **Table 3**, LOD values for the fragrance allergens ranged from 0.0118 to 0.0604 μg g^{-1} (excluding farnesol, 0.700 μg g^{-1}), for preservatives, these values were between 0.0053 and 0.0595 μg g^{-1} (excluding IPBC) and for plasticizers and musks LODs values ranged from 0.0026 to 0.1200 (excluding DIHP).

Therefore, the proposed micro-MSPD method using a vial instead of a mortar for the disruption and dispersion step can be considered suitable for the determination of fragrance allergens, preservatives, musks, and plasticizers in cosmetic and personal care products. It is highly recommended to decrease losses of most volatile fragrances such as pinene and limonene during sample preparation. For these compounds, the increase of temperature in the mortar disruption step is unfavorable for their quantitative extraction, whereas for in-vial disruption the generated heat is lower, and the most volatile compounds can be extracted lossless; also, in-vial disruption reduces extraction steps, providing a quicker extraction procedure.

Table 4. Recoveries of fragrance allergens, preservatives, plasticizers and musks in a leave-on sample (regenerating cream) fortified at three concentration levels, analyzed by the proposed method µMSPD-GC-MS.

Fragrance Allergens	Recoveries (%, RSD)					
	2 $\mu g \cdot g^{-1}$		10 $\mu g \cdot g^{-1}$		20 $\mu g \cdot g^{-1}$	
	Mortar	Vial	Mortar	Vial	Mortar	Vial
Pinene	23.2 (6.4)	63.1 (3.9)	23.5 (0.24)	71.8 (13)	34.0 (0.87)	72.6 (4.3)
Limonene	51.7 (2.0)	73.8 (1.1)	56.7 (7.1)	79.5 (11)	57.8 (0.016)	78.6 (10)
Benzyl alcohol	97.3 (0.76)	97.3 (1.3)	98.6 (0.80)	90.4 (7.9)	113 (0.15)	110 (1.9)
Linalool	105 (0.67)	82.5 (0.11)	96.7 (10)	89.3 (14)	107 (0.17)	100 (13)
Methyl-2-octynoate	87.8 (1.7)	85.2 (2.9)	97.5 (10)	86.7 (14)	112 (0.90)	95.4 (11)
Citronellol	101 (4.0)	89.3 (11)	97.6 (8.1)	93.1 (13)	110 (0.53)	94.8 (10)
Citral	99.0 (3.7)	104 (1.8)	97.5 (14)	112 (14)	112 (1.1)	101 (10)
Geraniol	114 (1.6)	82.5 (7.7)	82.0 (5.6)	81.7 (7.6)	102 (0.14)	92.7 (10)
Cinnamal	90.5 (4.5)	87.8 (0.74)	91.5 (12)	84.5 (13)	104 (0.88)	96.2 (6.6)
Hydroxycitronellal	81.9 (1.7)	80.1 (0.60)	101 (11)	97.8 (2.9)	114 (0.052)	101 (11)
Anise alcohol	93.8 (4.5)	92.2 (2.6)	96.2 (13)	87.4 (13)	111 (0.67)	101 (6.3)
Cinnamyl alcohol	96.3 (5.6)	87.3 (13)	94.0 (9.6)	87.2 (15)	110 (0.83)	98.8 (13)
Eugenol	87.2 (5.2)	83.0 (4.0)	93.8 (8.7)	89.2 (15)	105 (0.96)	98.7 (12)
Methyleugenol	85.8 (0.15)	83.6 (3.1)	95.5 (11)	86.7 (14)	109 (1.3)	98.7 (8.4)
Isoeugenol	80.9 (15)	100 (12)	114 (9.6)	109 (14)	87.2 (1.0)	89.4 (9.1)
Coumarin	91.1 (0.61)	85.2 (2.7)	95.5 (14)	87.3 (11)	109 (0.23)	100 (2.7)
α-isomethyl ionone	83.6 (6.2)	86.3 (0.78)	95.7 (11)	89.6 (12)	108 (0.82)	101 (7.4)
Lilial®	84.5 (3.3)	83.2 (1.4)	97.0 (11)	88.8 (15)	110 (1.7)	98.2 (10)
Amyl cinnamal	89.3 (4.4)	89.5 (7.8)	94.3 (5.2)	85.8 (4.3)	111 (3.5)	95.9 (15)
Lyral®	99.3 (11)	83.0 (12)	104 (6.9)	94.3 (5.4)	114 (2.8)	95.4 (14)
Amylcinnamyl alcohol	108 (10)	95.2 (12)	104 (7.4)	95.9 (3.9)	112 (3.1)	102 (15)
Farnesol	<LOQ	<LOQ	104 (2.7)	97.1 (7.6)	109 (7.5)	86.8 (7.5)
Hexylcinnamal	87.7 (1.8)	107 (0.12)	107 (4.5)	103 (5.1)	115 (3.8)	97.3 (11)
Benzyl benzoate	88.7 (7.6)	90.8 (6.9)	98.3 (14)	90.8 (12)	111 (0.16)	104 (4.9)
Benzyl salicylate	105 (3.1)	109 (13)	97.9 (14)	90.7 (13)	112 (1.9)	106 (2.8)
Benzyl cinnamate	94.5 (1.8)	102 (0.27)	102 (15)	94.2 (12)	112 (10)	108 (1.6)

Table 4. *Cont.*

	Recoveries (%, RSD)					
	2 μg·g^{-1}		10 μg·g^{-1}		20 μg·g^{-1}	
	Mortar	Vial	Mortar	Vial	Mortar	Vial
Preservatives						
Bronidox	92.1 (1.3)	88.0 (4.4)	95.6 (12)	87.3 (13)	108 (0.89)	100 (10)
PhEtOH	96.0 (3.8)	91.6 (0.87)	102 (12)	92.1 (13)	109 (0.91)	97.1 (11)
MeP	95.5 (4.2)	90.2 (6.4)	97.3 (11)	93.6 (15)	107 (1.7)	96.6 (11)
BHA	72.8 (6.6)	80.2 (1.2)	104 (9.4)	104 (13)	99.3 (0.53)	97.7 (7.3)
BHT	82.3 (0.16)	81.4 (0.33)	116 (0.83)	115 (12)	108 (0.67)	105 (6.9)
EtP	96.7 (10)	83.9 (15)	103 (13)	95.2 (14)	112 (0.36)	102 (10)
iPrP	100 (4.7)	91.0 (6.5)	100 (10)	93.2 (14)	109 (1.1)	98.2 (11)
PrP	111 (5.2)	87.5 (14)	99.3 (9.6)	97.5 (7.8)	109 (2.0)	94.3 (13)
IPBC	106 (15)	83.0 (12)	88.4 (1.6)	101 (4.5)	113 (4.0)	89.0 (16)
iBuP	113 (9.4)	92.6 (14)	103 (12)	95.2 (2.2)	111 (1.4)	98.5 (11)
BuP	96.8 (7.9)	82.0 (6.7)	99 (9.2)	88.9 (2.7)	110 (2.5)	95.2 (15)
Triclosan	109 (14)	112 (3.4)	100 (13)	107 (9.1)	111 (11)	108 (12)
BzP	105 (14)	92.6 (1.5)	111 (13)	111 (6.8)	117 (11)	111 (8.4)
Plasticizers						
DMA	93.9 (7.0)	105 (12)	116 (9.8)	108 (8.5)	103 (0.60)	97.5 (3.1)
DEA	87.5 (0.038)	81.4 (2.8)	95.4 (15)	87.5 (12)	111 (0.22)	100 (5.2)
DMP	81.5 (3.6)	83.3 (0.53)	98.2 (15)	90.5 (9.4)	109 (0.77)	102 (0.33)
DEP	79.3 (5.2)	83.1 (0.81)	96.1 (13)	87.2 (12)	110 (0.36)	101 (2.8)
DIBP	83.1 (1.2)	95.0 (2.1)	98.4 (14)	88.7 (14)	111 (0.88)	101 (3.2)
DBP	91.0 (6.5)	94.8 (3.3)	100 (14)	92.1 (8.8)	114 (2.5)	110 (3.6)
DMEP	90.3 (6.8)	103 (7.2)	107 (12)	99.2 (12)	114 (3.7)	108 (0.82)
DPP	96.8 (11)	98.3 (0.40)	101 (14)	96.3 (10)	110 (6.6)	107 (2.6)
BBP	89.7 (3.5)	83.7 (0.35)	92.0 (16)	86.7 (8.9)	103 (0.34)	96.9 (4.2)
DEHA	83.9 (7.8)	84.5 (2.7)	87.8 (14)	85.2 (8.5)	86.4 (0.87)	95.0 (0.76)

Table 4. *Cont.*

Plasticizers	Recoveries (%, RSD)					
	2 μg·g^{-1}		10 μg·g^{-1}		20 μg·g^{-1}	
	Mortar	Vial	Mortar	Vial	Mortar	Vial
DIHP	104 (5.3)	102 (1.1)	106 (6.3)	88.4 (6.0)	96.9 (6.2)	93.7 (5.1)
DEHP	98.1 (3.6)	81.1 (0.73)	93.3 (13)	92.4 (4.8)	101 (8.8)	96.4 (3.1)
DCHP	88.5 (2.4)	92.0 (5.1)	93.0 (12)	87.5 (6.4)	105 (2.4)	102 (3.4)
DPhP	84.9 (4.6)	84.4 (1.3)	90.5 (0.61)	84.3 (8.6)	102 (0.29)	96.9 (4.2)
DNOP	87.1 (1.2)	88.4 (2.8)	89.0 (15)	84.1 (9.2)	103 (3.6)	98.5 (2.2)
Musks						
Cashmeran	87.4 (2.0)	82.5 (9.5)	98.0 (13)	89.1 (13)	110 (1.5)	103 (7.2)
Celestolide	81.8 (5.7)	82.9 (0.80)	94.6 (9.9)	87.4 (3.1)	109 (1.9)	96.9 (9.0)
Phantolide	86.3 (4.9)	90.4 (0.67)	102 (10)	94.3 (3.4)	115 (3.3)	102 (11)
Ambrette	86.8 (9.3)	83.7 (14)	93.8 (0.23)	104 (7.1)	115 (6.1)	91.5 (6.2)
Traseolide	88.1 (7.6)	90.7 (3.3)	99.4 (9.4)	91.0 (4.5)	114 (2.6)	97.9 (10)
Galaxolide	88.9 (0.27)	94.7 (0.52)	103 (15)	95.3 (13)	114 (1.4)	105 (3.3)
Xylene	81.1 (12)	82.1 (5.6)	68.3 (2.1)	79.1 (2.6)	93.3 (3.1)	80.0 (6.2)
Tonalide	83.1 (1.4)	88.4 (0.72)	87.5 (14)	83.1 (8.2)	101 (0.92)	96.4 (1.4)
Moskene	89.8 (13)	86.1 (15)	93.5 (7.3)	83.2 (4.4)	114 (3.7)	94.6 (15)
Tibetene	103 (8.1)	109 (3.8)	100 (14)	93.0 (13)	115 (3.6)	111 (2.6)
Ambrettolide	96.0 (5.6)	111 (7.9)	106 (1.1)	107 (12)	113 (3.0)	108 (8.8)
Ketone	101 (14)	109 (6.1)	104 (10)	97.9 (15)	114 (5.9)	109 (6.9)

Table 5. Recoveries of fragrance allergens, preservatives, plasticizers and musks in a rinse-off sample (shampoo) fortified at three concentration levels, analyzed by the proposed method µMSPD-GC-MS.

Fragrance Allergens	Recoveries (%, RSD)					
	2 µg·g^{-1}		10 µg·g^{-1}		20 µg·g^{-1}	
	Mortar	Vial	Mortar	Vial	Mortar	Vial
Pinene	4.08 (12)	20.8 (0.59)	5.13 (0.38)	36.5 (1.2)	9.5 (6.9)	36.8 (9.4)
Limonene	28.2 (11)	64.7 (2.1)	28.1 (7.5)	75.8 (15)	38.9 (5.7)	65.9 (14)
Benzyl alcohol	113 (9.3)	90.8 (13)	85.7 (1.7)	92.2 (1.5)	105 (8.7)	102 (8.9)
Linalool	81.3 (9.3)	114 (6.9)	85.4 (3.6)	109 (2.5)	95.5 (14)	88.7 (0.53)
Methyl-2-octynoate	92.4 (2.8)	83.2 (5.2)	94.0 (6.7)	101 (5.6)	106 (11)	88.5 (15)
Citronellol	108 (1.4)	114 (5.0)	90.9 (5.0)	99.1 (3.8)	100 (14)	102 (6.8)
Citral	100 (3.0)	107 (10)	83.3 (6.4)	103 (7.0)	103 (12)	97.7 (4.3)
Geraniol	109 (4.0)	94.9 (13)	90.4 (11)	102 (3.4)	93.9 (13)	92.6 (0.44)
Cinnamal	105 (3.6)	89.1 (11)	88.5 (5.3)	94.2 (0.88)	103 (7.2)	96.0 (15)
Hydroxycitronellal	100 (14)	82.1 (5.0)	80.1 (2.7)	83.7 (4.7)	89.9 (12)	87.6 (3.7)
Anise alcohol	112 (8.5)	90.1 (15)	86.6 (2.4)	97.1 (0.76)	101 (10)	91.7 (9.3)
Cinnamyl alcohol	113 (7.9)	83.4 (15)	87.6 (4.3)	97.7 (0.40)	97.9 (13)	95.5 (13)
Eugenol	106 (3.5)	88.6 (13)	87.5 (1.9)	99.1 (1.2)	98.6 (10)	92.1 (15)
Methyleugenol	110 (10)	94.3 (1.5)	96.4 (1.2)	102 (0.33)	104 (4.4)	95.9 (10)
Isoeugenol	87.9 (10)	83.8 (6.9)	107 (2.6)	119 (0.42)	96.2 (5.0)	92.7 (10)
Coumarin	111 (12)	90.1 (3.8)	89.7 (1.7)	97.1 (3.5)	104 (1.7)	97.8 (7.9)
α-isomethyl ionone	110 (10)	97.4 (0.76)	94.4 (0.84)	98.4 (1.2)	103 (4.3)	93.4 (6.5)
Lilial®	106 (12)	91.7 (2.3)	88.2 (0.10)	93.3 (4.1)	98.9 (5.3)	88.3 (12)
Amyl cinnamal	112 (5.8)	106 (9.2)	102 (3.6)	109 (4.1)	108 (11)	102 (16)
Lyral®	105 (1.7)	112 (9.0)	89.2 (1.2)	99.4 (2.3)	98.8 (11)	82.6 (4.9)
Amylcinnamyl alcohol	113 (8.3)	118 (5.5)	98.8 (1.8)	114 (1.0)	112 (7.7)	113 (12)
Farnesol	<LOQ	<LOQ	97 (3.2)	111 (6.8)	95.0 (15)	108 (8.4)
Hexylcinnamal	108 (1.2)	105 (5.5)	105 (3.1)	111 (4.7)	108 (9.5)	104 (11)
Benzyl benzoate	111 (11)	98.4 (1.5)	92.3 (1.6)	103 (0.42)	104 (3.1)	97.6 (5.6)

Table 5. *Cont.*

Fragrance Allergens	Recoveries (%, RSD)					
	2 μg·g^{-1}		10 μg·g^{-1}		20 μg·g^{-1}	
	Mortar	Vial	Mortar	Vial	Mortar	Vial
Benzyl salicylate	116 (1.2)	113 (3.7)	80.8 (0.51)	102 (4.5)	111 (8.4)	104 (10)
Benzyl cinnamate	102 (12)	102 (2.1)	102 (5.4)	113 (3.9)	119 (4.4)	116 (3.3)
Preservatives						
Bronidox	104 (2.8)	90.4 (11)	94.4 (3.2)	97 (5.3)	101 (7.7)	95.9 (16)
PhEtOH	112 (10)	90.5 (2.1)	91.7 (3.1)	94 (5.5)	98.4 (12)	92.6 (11)
MeP[a]	n.c	n.c	n.c	n.c	n.c	n.c
BHA	106 (12)	98.4 (1.3)	105 (5.7)	110 (1.1)	100 (3.1)	100 (8.3)
BHT[a]	n.c	n.c	n.c	n.c	n.c	n.c
EtP	112 (1.0)	103 (1.7)	102 (2.5)	102 (0.32)	110 (3.9)	108 (10)
iPrP	118 (3.2)	114 (15)	102 (1.1)	110 (3.4)	111 (6.8)	113 (7.4)
PrP[a]	n.c	n.c	n.c	n.c	n.c	n.c
IPBC	113 (8.7)	97.7 (14)	103 (8.0)	117 (0.50)	111 (16)	95.4 (7.9)
iBuP	94.1 (15)	103 (2.4)	104 (2.9)	109 (2.3)	111 (0.13)	113 (8.4)
BuP	115 (1.0)	110 (1.6)	108 (2.7)	114 (2.4)	116 (1.6)	113 (10)
Triclosan	87.0 (6.9)	84.4 (6.1)	81.7 (6.7)	91 (7.8)	118 (6.4)	113 (11)
BzP	87.3 (11)	104 (9.5)	81.4 (2.0)	112 (1.7)	104 (12)	103 (11)
Plasticizers						
DMA	98.6 (5.5)	95.1 (2.7)	104 (0.65)	72.4 (0.65)	104 (1.3)	96.6 (9.4)
DEA	101 (5.5)	81.3 (6.9)	94.8 (1.6)	92.8 (1.1)	103 (4.0)	91.8 (11)
DMP	109 (12)	87.9 (1.5)	95.4 (2.7)	94.2 (0.72)	103 (1.4)	95.0 (7.6)
DEP	105 (7.0)	92.2 (0.34)	102 (2.5)	100 (0.20)	101 (3.2)	100 (8.6)
DIBP	115 (4.9)	93.3 (2.7)	94.1 (2.4)	102 (0.64)	98.9 (4.6)	102 (10)
DBP	117 (12)	109 (0.69)	91.5 (2.0)	108 (1.7)	105 (5.9)	110 (9.3)
DMEP	112 (10)	100 (1.6)	112 (7.9)	119 (4.9)	113 (2.3)	112 (8.7)
DPP	113 (11)	88.4 (4.1)	94.7 (4.2)	107 (0.56)	114 (3.9)	115 (5.9)

Table 5. *Cont.*

Plasticizers	Recoveries (%, RSD)					
	2 µg·g^{-1}		10 µg·g^{-1}		20 µg·g^{-1}	
	Mortar	Vial	Mortar	Vial	Mortar	Vial
BBP	114 (5.6)	115 (2.2)	101 (5.4)	108 (2.4)	117 (3.0)	119 (0.20)
DEHA	96.9 (9.1)	105 (7.0)	101 (6.6)	108 (8.5)	112 (1.0)	107 (2.8)
DIHP	98.5 (1.4)	102 (7.9)	98.1 (1.7)	102 (3.4)	99.2 (6.8)	113 (7.3)
DEHP	116 (2.3)	93.4 (3.5)	103 (4.5)	101 (4.6)	109 (4.4)	103 (5.2)
DCHP	116 (8.7)	107 (0.94)	107 (6.9)	107 (1.5)	112 (10)	114 (0.43)
DPhP	116 (11)	105 (1.5)	95.9 (6.9)	104 (3.2)	113 (1.1)	108 (2.2)
DNOP	118 (4.4)	87.8 (8.3)	96.1 (6.5)	106 (0.48)	111 (2.3)	120 (4.4)
Musks						
Cashmeran	112 (8.9)	101 (1.8)	113 (0.77)	99 (1.2)	103 (4.0)	96.8 (11)
Celestolide	113 (12)	115 (1.1)	114 (0.99)	107 (0.47)	104 (4.0)	101 (6.8)
Phantolide	109 (10)	107 (3.4)	113 (1.4)	111 (4.2)	102 (10)	103 (14)
Ambrette	118 (6.4)	104 (10)	118 (6.7)	118 (11)	100 (10)	106 (10)
Traseolide	113 (10)	104 (7.3)	95.2 (1.4)	102 (3.8)	103 (9.2)	100 (12)
Galaxolide	113 (13)	100 (2.6)	106 (2.0)	98.9 (0.011)	101 (4.0)	97.2 (8.0)
Xylene	118 (2.9)	111 (8.2)	101 (5.6)	88.9 (14)	104 (7.1)	114 (4.3)
Tonalide	117 (16)	113 (2.6)	90.7 (0.77)	89.8 (1.3)	101 (5.4)	98.0 (7.6)
Moskene	111 (15)	96.7 (3.6)	109 (4.0)	105 (10)	103 (16)	96.0 (7.3)
Tibetene	112 (12)	108 (5.7)	111 (1.7)	99.0 (6.2)	104 (10)	100 (16)
Ambrettolide	115 (3.8)	111 (3.5)	99.3 (1.3)	101 82.7)	104 (6.1)	108 (14)
Ketone	79.6 (4.5)	114 (13)	93.5 (0.75)	109 (4.0)	117 (8.0)	113 (11)

[a] n.c: not calculated since initial sample concentration is higher than the spiked level. MeP, BHT, and PrP: 22, 21, and 10 µg·g^{-1}, respectively.

Table 6. Recovery study in different cosmetic matrices. Spike level: 10 $\mu g \cdot g^{-1}$.

Fragrance Allergens	Recoveries (%, RSD)					
	S3 [a]		S6 [a]		S8 [a]	
	Mortar	Vial	Mortar	Vial	Mortar	Vial
Pinene	4.2 (7.3)	72.1 (1.5)	11.2 (3.1)	74.0 (0.013)	21.2 (11)	74.3 (4.1)
Limonene	35.4 (5.6)	90.3 (1.7)	81.3 (3.1)	77.0 (2.1)	57.1 (2.5)	93.0 (0.87)
Benzyl alcohol	115 (6.9)	109 (0.19)	114 (4.2)	91.2 (10)	113 (0.51)	116 (0.38)
Linalool	102 (4.5)	117 (0.021)	n.c	n.c	n.c	n.c
Methyl-2-octynoate	106 (10.4)	93.0 (3.4)	94.9 (8.6)	81.3 (9.7)	103 (3.7)	113 (6.1)
Citronellol	89.8 (13)	101 (2.8)	110 (3.8)	100 (2.2)	90.0 (2.1)	100 (2.8)
Citral	113 (7.1)	105 (6.7)	115 (5.0)	94.2 (4.0)	107 (2.9)	108 (8.1)
Geraniol	80.4 (13)	93.4 (1.9)	107 (7.8)	92.4 (4.1)	91.2 (8.1)	98.2 (9.7)
Cinnamal	98.8 (9.4)	103 (2.8)	106 (7.4)	98.2 (4.6)	101 (2.4)	108 (2.5)
Hydroxycitronellal	108 (12)	102 (6.8)	93.6 (6.3)	81.0 (7.4)	112 (6.1)	111 (6.8)
Anise alcohol	98.3 (13)	101 (4.2)	95.4 (5.2)	96.1 (6.2)	83.3 (6.6)	104 (0.041)
Cinnamyl alcohol	81.7 (15)	96.9 (6.7)	112 (11)	91.4 (8.8)	106 (8.7)	109 (3.5)
Eugenol	100 (11)	95.4 (4.2)	115 (10)	92.1 (3.6)	101 (6.1)	109 (5.9)
Methyleugenol	100 (8.3)	110 (0.45)	96.4 (6.5)	96.1 (4.9)	100 (3.5)	106 (3.7)
Isoeugenol	102 (9.9)	93.0 (2.9)	114 (15)	82.5 (5.3)	86.2 (3.3)	95.2 (0.45)
Coumarin	107 (11)	118 (0.62)	95.8 (2.1)	81.0 (1.5)	91.3 (1.3)	97.1 (0.52)
α-isomethyl ionone	98.4 (7.5)	104 (1.9)	96.8 (3.7)	94.6 (0.24)	98.8 (3.1)	103 (2.5)
Lilial®	99.2 (8.6)	98.1 (1.2)	97.2 (4.5)	85.0 (0.055)	96.3 (3.7)	102 (4.2)
Amyl cinnamal	106 (10)	107 (0.82)	105 (8.1)	96.4 (3.6)	98.7 (4.5)	106 (4.2)
Lyral®	115 (7.3)	98.0 (1.5)	85.8 (12)	82.0 (11)	112 (6.7)	114 (7.5)
Amylcinnamyl alcohol	104 (10)	98.3 (6.7)	109 (9.7)	96.5 (9.4)	111 (4.3)	111 (9.1)
Farnesol	92.3 (13)	95.0 (0.29)	97.2 (1.8)	95.9 (13)	109 (8.5)	106 (12)
Hexylcinnamal	107 (14)	119 (0.29)	n.c	n.c	105 (5.9)	112 (5.9)
Benzyl benzoate	102 (9.0)	105 (0.43)	101 (2.4)	95.4 (1.2)	107 (3.8)	113 (5.1)
Benzyl salicylate	112 (10)	115 (6.9)	n.c	n.c	113 (3.5)	114 (4.3)
Benzyl cinnamate	115 (6.7)	116 (0.14)	113 (9.0)	112 (0.40)	113 (6.1)	115 (4.8)

Table 6. *Cont.*

	Recoveries (%, RSD)					
	S3 [a]		S6 [a]		S8 [a]	
	Mortar	Vial	Mortar	Vial	Mortar	Vial
Preservatives						
Bronidox	93.9 (7.5)	101 (2.7)	106 (5.2)	108 (0.94)	105 (0.72)	95.0 (5.2)
PhEtOH	n.c	n.c	n.c	n.c	n.c	n.c
MeP	112 (11)	100 (7.7)	n.c	n.c	n.c	n.c
BHA	99.1 (9.6)	94.2 (0.89)	109 (6.2)	83.2 (0.91)	93.1 (3.1)	102 (2.7)
BHT	98.4 (3.1)	92.0 (0.040)	n.c	n.c	91.2 (3.4)	100 (3.2)
EtP	108 (7.7)	92.0 (9.8)	n.c	n.c	n.c	n.c
iPrP	105 (11)	114 (8.5)	114 (6.3)	115 (1.2)	94.9 (3.9)	103 (3.8)
PrP	115 (8.7)	115 (1.14)	n.c	n.c	n.c	n.c
IPBC	106 (14)	84.2 (10)	101 (7.4)	113 (15)	110 (18)	114 (10)
iBuP	105 (13)	111 (5.3)	n.c	n.c	112 (9.6)	106 (1.4)
BuP	103 (14)	107 (0.79)	n.c	n.c	107 (13)	103 (6.8)
Triclosan	119 (5.8)	96.0 (3.0)	114 (3.3)	106 (13)	104 (8.8)	110 (3.5)
BzP	96.0 (14)	108 (7.9)	95.1 (2.8)	116 (9.0)	110 (10)	102 (3.5)
Plasticizers						
DMA	105 (8.1)	105 (1.1)	97.0 (3.7)	84.0 (2.8)	95.1 (0.59)	98.2 (3.1)
DEA	108 (6.5)	100 (2.9)	104 (5.2)	96.1 (2.4)	105 (4.6)	113 (5.4)
DMP	96.4 (7.6)	104 (1.8)	95.0 (5.8)	88.3 (2.0)	95.2 (0.75)	101 (1.5)
DEP	97.8 (7.4)	107 (0.41)	n.c	n.c	n.c	n.c
DIBP	97.6 (10)	104 (0.24)	100 (2.5)	88.2 (1.5)	96.4 (2.5)	102 (3.1)
DBP	109 (8.4)	115 (1.1)	108 (4.4)	98.7 (2.1)	106 (3.2)	112 (4.1)
DMEP	113 (8.7)	119 (5.8)	98.2 (7.0)	103 (3.0)	107 (7.9)	114 (4.9)
DPP	108 (7.3)	108 (0.93)	112 (3.7)	101 (0.82)	114 (2.7)	115 (2.2)
BBP	97.3 (8.1)	97 (1.9)	109 (3.6)	86.0 (0.94)	103 (4.8)	110 (0.74)
DEHA	112 (6.9)	83 (3.3)	83.1 (4.1)	80.2 (1.7)	96.4 (4.9)	103 (0.70)
DIHP	113 (6.6)	115 (4.6)	108 (7.3)	107 (15)	101 (10)	89.0 (1.5)

Table 6. *Cont.*

Plasticizers	Recoveries (%, RSD)					
	S3 [a]		S6 [a]		S8 [a]	
	Mortar	Vial	Mortar	Vial	Mortar	Vial
DEHP	114 (13)	93 (1.3)	99.2 (4.4)	90.3 (2.2)	105 (2.1)	112 (2.1)
DCHP	113 (14)	96 (0.80)	94.0 (8.6)	88.5 (1.5)	97.2 (8.5)	97.2 (7.5)
DPhP	112 (7.6)	102 (0.13)	115 (3.0)	92.0 (5.9)	102 (3.1)	108 (3.1)
DNOP	102 (8.1)	114 (0.88)	113 (2.8)	100 (0.31)	114 (1.5)	113 (2.4)
Musks						
Cashmeran	98.4 (8.6)	96.0 (0.32)	93.0 (7.0)	88.3 (1.6)	97.2 (2.4)	103 (2.5)
Celestolide	102 (8.5)	103 (1.0)	93.5 (6.9)	93.5 (1.6)	98.0 (4.8)	105 (5.0)
Phantolide	100 (8.1)	102 (1.1)	96.4 (4.4)	93.9 (1.0)	101 (4.8)	107 (4.4)
Ambrette	92.0 (13)	84.2 (13)	113 (6.3)	112 (15)	112 (10)	113 (11)
Traseolide	98.8 (10)	100 (2.3)	105 (7.7)	101 (0.10)	105 (5.3)	113 (4.9)
Galaxolide	98.0 (10)	102 (0.72)	n.c	n.c	n.c	n.c
Xylene	88.2 (9.2)	--	111 (8.9)	--	86.0 (5.5)	97.4 (7.4)
Tonalide	98.4 (10)	98.0 (0.83)	81.0 (2.6)	84.0 (2.2)	n.c	n.c
Moskene	93.0 (12)	89.1 (11)	109 (11)	103 (15)	98.2 (8.1)	103 (8.6)
Tibetene	107 (10)	105 (4.4)	111 (11)	106 (7.8)	104 (7.1)	109 (7.9)
Ambrettolide	114 (13)	101 (0.98)	94.4 (10)	86.2 (4.8)	80.1 (10)	93.2 (3.5)
Ketone	109 (11)	99.0 (7.4)	109 (13)	114 (3.7)	108 (9.5)	115 (10)

[a] See initial concentration in Table 7. n.c: not calculated since initial sample concentration is higher than the spiked level.

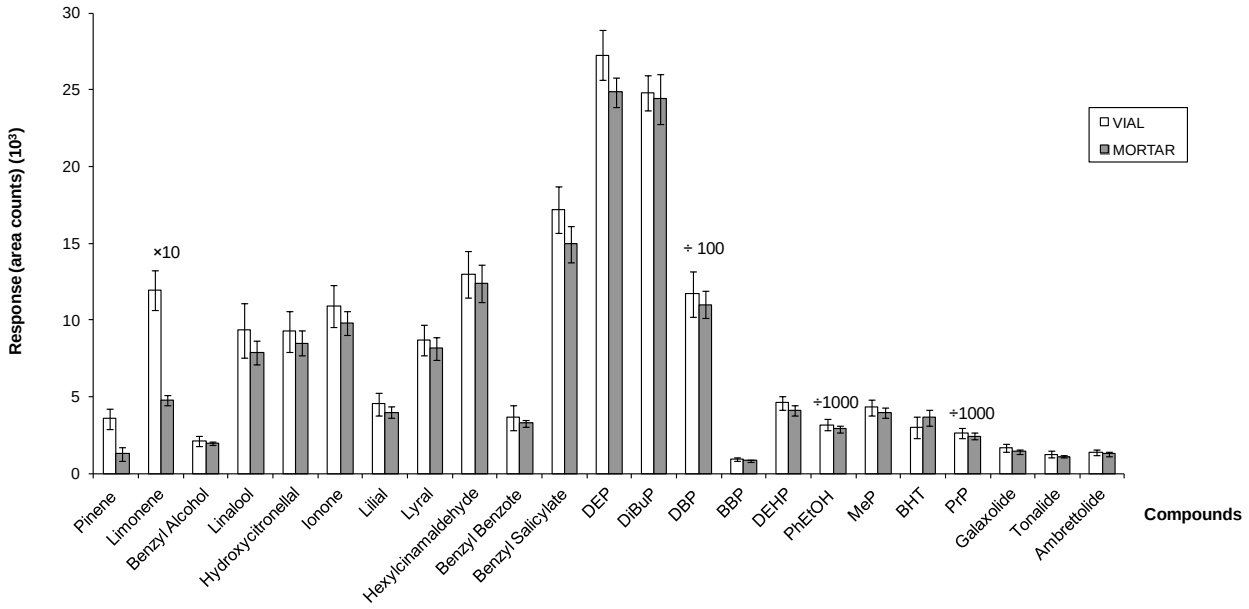

Figure 3. Comparative results between in vial or mortar μ-MSPD for a leave-on sample (hands cream).

3.3. Application to Real Samples

Finally, the validated method was applied to the analysis of 18 real cosmetic and personal care products, including five rinse-off (shower gel, shampoos and baby liquid soap) and 13 leave-on (sunblock, after sun, body milk, hands cream, deodorants, among others) products, which represent a wide variety of personal care products. Results are shown in **Table 7**. Forty-eight of the 66 targets were found in the samples, with a minimum of 12 and a maximum of 28 compounds in each sample, at global concentrations ranging from 0.043% to 1.6%. It is worthy to note that the sample containing more targets is a baby body care lotion (sample S16).

3.3.1. Fragrance Allergens

Twenty-two of the 26 fragrance allergens were found in the analyzed samples. Linalool was detected in 83% of the samples at concentration values up to 0.1%. Also limonene, coumarin, benzyl alcohol, and benzyl salicylate were found in many samples at concentrations below 800 $\mu g \cdot g^{-1}$. Other fragrance allergens were detected in 2–12 samples. It is remarkable the presence of farnesol at high concentrations (>0.2%) in two leave-on samples (S12 and S13). Regarding the number of compounds per sample, three leave-on samples (S7, S16 and S18) contained 15 target allergens. In the other samples, the number of compounds was 3–12.

3.3.2. Preservatives

Phenoxyethanol was the most frequent found preservative (83% of the samples) at concentration values higher than 0.1% (1000 $\mu g \cdot g^{-1}$) in nine samples. In sample S11, phenoxyethanol concentration (1.5%) surpassed the maximum concentration permitted by European regulation (see **Table 1**). In the case of parabens, six of the seven targets were found in the analyzed samples. The most common was PrP (67%) at 0.1% in two leave-on samples (S9 and S10). Other parabens, MeP, EtP, BuP, iBuP, and iPrP, were found in 11, seven, five, four and one samples, respectively. Triclosan was detected in two samples, at very high concentration in a deodorant (S13), reaching the limit established by the European legislation (0.3%). BHT, IPBC, and BHA were detected in 13, three and one sample, respectively. The highest number of preservatives was found in S5 and S6, with eight targets. It should be noted that S5 is a care cream intended for babies. A hand cream (S11) does not comply with European restrictions regarding PhEtOH (>1%) and total paraben concentration (> 0.8%).

3.3.3. Plasticizers

DEP was found in all analyzed samples at concentration levels below 432 $\mu g \cdot g^{-1}$, except in the deodorant S14 (1539 $\mu g \cdot g^{-1}$). Two banned phthalates (DBP and DEHP) were detected in 13 and 11 samples, respectively, at concentrations between 0.05 and 4.8 $\mu g \cdot g^{-1}$. It should be noted that sample S11 (a hand cream) contained a DBP concentration >0.1%. In the other personal care products, the number of plasticizers was 1–6, highlighting the presence of DEHA at very high concentration (1.6%) in make-up (leave-on sample, S17).

Table 7. Analysis of target compounds in rinse-off and leave-on cosmetic samples (% w/w ×10^4) [a].

	Rinse-off samples[b]					*Leave-on samples*[b]												
Fragrance allergens	***S1***	***S2***	***S3***	***S4***	***S5***	***S6***	***S7***	***S8***	***S9***	***S10***	***S11***	***S12***	***S13***	***S14***	***S15***	***S16***	***S17***	***S18***
Pinene		0.777		1.18			2.24				1.51		0.972	0.396	1.40	0.881	0.426	0.0800
Limonene	266	148		283		0.446	199		152		11.0	1.31	163	222	53.2	14.3	2.73	6.38
Benzyl alcohol	4.00				3.32	3.06	7.55		2.50	2.93	31.2	3.76	17.9			6.14	0.698	27.5
Linalool	666	84.6		172		27.3	358	17.2	369	101	161	252	178	1024	255	228		26.4
Methyl-2-octynoate	60.4							105	24.7				72.0					
Citronellol	25.0						79.6			1.45	45.3	234		63.1		58.0	7.75	13.2
Citral				13.6			8.84		10.5	6.28					6.50			
Geraniol	90.2	19.1		44.5			167			2.83	23.1	10.3			37.2	41.1		17.3
Cinnamal														4.73		1.14		
Hydroxycitronellal			2.08			0.212	33.1				57.4			1.29		18.3	52.0	1.00
Anise alcohol						0.155		23.6										
Cinnamyl alcohol																12.4	9.23	6.42
Eugenol						2.67						1.66	31.8	40.5			3.63	0.114
Coumarin	30.8	3.44	6.89	9.55		0.460	11.8		16.6			113	21.0	557	3.25		1.79	20.3
α-isomethyl ionone	3.32					0.297	31.5		48.5		36.5			136		89.9	167	31.8
Lilial®		7.83	1.36			0.843	30.8		172		14.0	331	491	2.79	233	82.4		
Amyl cinnamal		116								7.34								
Lyral®							108				211					58.1	41.1	10.7
Farnesol												2924	2188					
Hexylcinnamal						12.9	29.6		27.4		115	393	885	4.37	0.475	60.2		74.5
Benzyl benzoate		0.316		5.88	0.380	0.452	5.66				7.63	1.53		0.789	1.28	4.21	14.1	8.28
Benzyl salicylate		2.48		524	0.355	12.4	4.29		217			111	770	0.642	1.70	486	19.9	10.3

Table 7. *Cont.*

Preservatives	***S1***	***S2***	***S3***	***S4***	***S5***	***S6***	***S7***	***S8***	***S9***	***S10***	***S11***	***S12***	***S13***	***S14***	***S15***	***S16***	***S17***	***S18***
PhEtOH	14.2		15.3	1.30	2009	1395	2455	3609	4017	1.45	15530		0.383		17.3	1993	1877	1849
MeP[c]		4.65	3.22		284	1244	654	789	887		1484				2.74	540		1350
BHA						2.78												
BHT	0.544	1.98		0.105	0.0430	12.1	186			2996		20.3	9.93	5.01	625	0.237	25.2	
EtP[c]					89.7	135	202	203							5.19	119		15.46
iPrP[c]					144													
PrP[c]		1.61	0.374		60.1	470	1.45	364	262	1146	7660				1.76	62.4		588
IPBC			0.504	40.2								5.94						
iBuP[c]			0.258		46.7	57.3										64.5		
BuP[c]			1.17		91.0	146									0.280	122		
Triclosan													2794	1.21				
Plastizicers	***S1***	***S2***	***S3***	***S4***	***S5***	***S6***	***S7***	***S8***	***S9***	***S10***	***S11***	***S12***	***S13***	***S14***	***S15***	***S16***	***S17***	***S18***
DEP	432	153	0.888	0.190	0.316	109	39.4	46.0	0.118	0.378	20.4	0.737	1.88	1539	0.254	40.5	303	0.300
DIBP		0.138							0.337	0.420	26.4		0.292	0.546				
DBP	0.409	0.0722	0.0511			0.509	0.194	0.340	0.299	0.549	1434		2.24	0.575	1.37			1.24
DEHA		10.4															16387	
DEHP	0.640	0.731	0.168	0.553	0.800		0.986				4.78	1.24	1.02	0.818		1.78		
DCHP													0.196					
DPhP										8.48								
DNOP													0.444	0.0932				
Musks	***S1***	***S2***	***S3***	***S4***	***S5***	***S6***	***S7***	***S8***	***S9***	***S10***	***S11***	***S12***	***S13***	***S14***	***S15***	***S16***	***S17***	***S18***
Celestolide	1.23						0.598	0.110										
Phantolide	0.320																	
Galaxolide	625	0.379		0.0820		114	93.2	128		0.119	3.90	199	0.120	0.844		86.8	211	
Xylene																3.14		
Tonalide	244							20.8			3.54			0.153		26.3		
Ambrettolide			16.0	34.7							10.8							
Ketone							33.4									1368		

[a] Equivalent to $\mu g \cdot g^{-1}$. [b] S1: Shower gel; S2, S3: Shampoos, S4, S5: Babies liquid soap; S6: Sunblock; S7: Aftersun; S8, S9: Body milk; S10: Lipstick; S11: Hands cream; S12, S13, S14: Deodorants; S15, S16: Baby moisturising lotions; S17: Makeup; S18: Moisturising milk. [c] Parabens concentration expressed as acid (% w/w × 10^4).

3.3.4. Musks

Galaxolide was found in 72% of the samples at concentrations below 625 $\mu g \cdot g^{-1}$. Celestolide and ambrettolide were found in 17% of the samples at concentration levels between 0.11 and 35 $\mu g \cdot g^{-1}$. The restricted musks tonalide, ketone, xylene and phantolide were detected in at least one sample (tonalide in five samples); at concentrations fulfilling the EU limits, with the exception of musk ketone, found at >0.042% in sample S16, a baby moisturizing lotion.

4. CONCLUSIONS

A micro-MSPD-GC-MS method has been proposed for the determination of four families of compounds extensively used in cosmetics and personal care product formulations: fragrance allergens, preservatives, plasticizers, and synthetic musks. This study included 66 chemicals subjected to restrictions according European legislation. We compared the performance of two micro-MSPD procedures for the extraction of the targets, performing the sample disruption in mortar and in vial. The proposed in-vial method allows analytes extraction in less than 5 minutes, providing a quick and low cost extraction procedure with lower losses of the more volatile compounds. The method was validated showing satisfactory linearity, sensitivity, accuracy, and precision, with recoveries higher than 90% and RSD values below 10%. Finally, the method was applied to real cosmetic samples including different matrices to demonstrate the method performance. Forty-eight of the 66 targets were detected in the analyzed samples. Several compounds were present at concentrations higher than 0.1%, and two of the samples did not comply with European requirements. The analyzed products intended for baby care contained similar or even higher numbers and concentrations of regulated compounds, highlighting the high number of preservatives, and the presence of musk ketone above the legal limit in one of these kinds of products.

Acknowledgments

This research was supported by project CTQ 2013-46545-P (Ministerio de Economía y Competitividad, Spain)

References

[1] European Union. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products (recast). Off. J. Eur. Union 2009, *L342*, 59–209.

[2] M.G. Kirchhof, G.C. de Gannes, Skin Ther. Lett., 18 (2013) 5.

[3] S. Polati, S. Gosetti, M.C. Gennaro, Preservatives in Cosmetics. Analytical Methods. In *Analysis of Cosmetic Products*; Salvador, A., Chisvert, A., Eds.; Elsevier: Amsterdam, The Netherland, 2007.

[4] P.D. Darbre, P.W. Harvey, J. Appl. Toxicol., 28 (2008) 561.

[5] G.J. Nohynek, C.J. Borgert, D. Dietrich, K.K. Rozman, Toxicol. Lett., 223 (2013) 295.

[6] J. Boberg, C. Taxvig, S. Christiansen, U. Hass, Reprod. Toxicol., 30 (2010) 301.

[7] ANSM. L'ANSM Recommande de Restreindre la Concentration de Phénoxyéthanol dans les Produits Cosmétiques Destinés aux Enfants de Moins de 3 ans—Point D'information. Available at: http://ansm.sante.fr/S-informer/Points-d-information-Points-d-information/L-ANSM-recommande-de-restreindre-la-concentration-de-phenoxyethanol-dans-les-produits-cosmetiques-destines-aux-enfants-de-moins-de-3-ans-Point-d-information (accessed on May 2014).

[8] L. Roosens, A. Covaci, H. Neels, Chemosphere, 69 (2007) 1540.

[9] G. Petersen, D. Rasmussen, K. Gustavson, DHI Water and Environment: Hørsholm, Denmark, 2007.

[10] A. Chisvert, A. Salvador, Perfumes in Cosmetics. Analytical Methods. In *Analysis of Cosmetic Products*; Salvador, A., Chisvert, A., Ed.; Elsevier: Amsterdam, The Netherland, 2007.

[11] N. Cabaleiro, I. de la Calle, C. Bendicho, I. Lavilla, Anal. Methods, 5 (2013) 323.

[12] N. Cabaleiro, I. de la Calle, C. Bendicho, I. Lavilla, TrAC Trends Anal. Chem., 57 (2014) 34.

[13] L.H. Wang, Anal. Methods, 5 (2013) 316.

[14] A.L. Capriotti, C. Cavaliere, A. Lagana, S. Piovesana, R. Samperi, TrAC Trends Anal. Chem., 43 (2013) 53.

[15] E.M. Kristenson, U.A.T Brinkman, L. Ramos, TrAC Trends Anal. Chem., 25 (2006) 96.

[16] G. Alvarez-Rivera, T. Dagnac, M. Lores, C. Garcia-Jares, L. Sanchez-Prado, J.P. Lamas, M. Llompart, J. Chromatogr. A, 1270 (2012) 41.

[17] L. Sanchez-Prado, G. Alvarez-Rivera, J.P. Lamas, M. Llompart, M. Lores, C. Garcia-Jares, Anal. Methods, 5 (2013) 416.

[18] L. Sanchez-Prado, G. Alvarez-Rivera, J.P. Lamas, M. Lores, C. Garcia-Jares, M. Llompart, Anal. Bioanal. Chem., 401 (2011) 3293.

[19] M. Llompart, M. Celeiro, J.P. Lamas, L. Sanchez-Prado, M. Lores, C. Garcia-Jares, J. Chromatogr. A, 1293 (2013) 10.

[20] M. Celeiro, E. Guerra, J.P. Lamas, M. Lores, C. Garcia-Jares, M. Llompart, J. Chromatogr. A, 1344 (2014) 1.

[21] L. Sanchez-Prado, J.P. Lamas, G. Alvarez-Rivera, M. Lores, C. Garcia-Jares, M. Llompart, J. Chromatogr. A, 1218 (2011) 5055.

[22] J.P. Lamas, L. Sanchez-Prado, C. Garcia-Jares, M. Lores, M. Llompart, J. Chromatogr. A, 1217 (2010) 8087.

[23] E. Becerril-Bravo, J.P. Lamas, L. Sanchez-Prado, M. Lores, C. Garcia-Jares, B. Jimenez, M. Llompart, Chemosphere, 81 (2010) 1378.

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1.4. PRESSURIZED LIQUID EXTRACTION-GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF FRAGRANCE ALLERGENS, MUSKS, PHTHALATES AND PRESERVATIVES IN BABY WIPES

Journal of Chromatography A. (Ed. Elsevier, Netherlands)
ISSN: 0021-9673

***J. Chromatogr. A., 1384 (2015) 9-21*. doi: 10.1016/j.chroma.2015.01.049**

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PRESSURIZED LIQUID EXTRACTION-GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF FRAGRANCE ALLERGENS, MUSKS, PHTHALATES AND PRESERVATIVES IN BABY WIPES

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ABSTRACT

Baby wipes and wet toilet paper are specific hygiene care daily products used on newborn and children skin. These products may contain complexes mixtures of harmful chemicals. A method based on pressurized liquid extraction (PLE) followed by gas chromatography-mass spectrometry (GC-MS) has been developed for the simultaneous determination of sixty-five chemical compounds (fragrance allergens, preservatives, musks, and phthalates) in wipes and wet toilet paper for children. These compounds are legislated in Europe according Regulation EC No 1223/2009, being twelve of them banned for their use in cosmetics, and one of them, 3-iodo-2-propynyl butylcarbamate (IPBC), is banned in products intended for children under 3 years. Also, propyl-, and butylparaben will be prohibited in leave-on cosmetic products designed for application on the nappy area of children under 3 years from April 2015. PLE is a fast, simple, easily automated technique, which permits to integrate a clean-up step during the extraction process reducing analysis time and stages. The proposed PLE-based procedure was optimized on real non-spiked baby wipe samples by means of experimental design to study the influence on extraction of parameters such as extraction solvent, temperature, extraction time, and sorbent type. Under the selected conditions, the method was validated showing satisfactory linearity, and intra-day, and inter-day precision. Recoveries were between 80-115% for most of the compounds with relative standard deviations (RSD) lower than 15%. Finally, twenty real samples were analyzed. Thirty-six of the target analytes were detected, highlighting the presence of phenoxyethanol in all analyzed samples at high concentration levels (up to 0.8%, 800 μg g^{-1}). Methyl paraben (MeP), and ethyl paraben (EtP) were found in 40-50% of the samples, and the recently banned isobutyl paraben (iBuP) and isopropyl paraben (iPrP), were detected in one and seven samples, respectively, at concentrations between 0.093-247 μg g^{-1}. In the case of phthalates, the forbidden phthalates dibutyl phtalate (DBP) and di(2-ethylhexyl)phthalate (DEHP) were also found in thirteen samples at low levels. Most samples contained fragrance allergens in many cases at high levels (up to 2400 μg g^{-1}) and three musks were detected in the samples. Excluding the banned compounds, all samples complied with the concentration limits established by the European Regulation although 25% of them did not fulfill the labeling requirements for fragrance allergens.

Keywords: fragrance allergens; preservatives; phthalates; musks; baby wipes; pressurized liquid extraction; GC-MS; personal care products (PCPs); cosmetics

1. INTRODUCTION

In last years, there is an increasing use of disposable wet tissues due to their commodity; makeup remover, suntan cream, deodorants or moisturizing lotions among others, are offered as wipes. Child specific care products such as baby wipes are usually employed for the cleansing of newborns, babies and children [1-3]. Children under three years are daily exposed to this product (up to sixteen units per day), mainly applied in the sensitive diaper zone including the genital area. This zone has a higher pH and is usually irritated due to the prolonged contact of the skin with urine and feces, which may damage the skin barrier and increase its permeability [4]. The skin barrier development in babies remains incomplete until 12 months of age, and the trans epidermal water loss in an infant is much higher than in an adult [5]. Therefore, skin is more susceptible to microbial and contaminants invasion. It has been reported that around 25% of babies develop atopic dermatitis, and 50% show napkin dermatitis [6,7]. In addition, babies and children are most susceptible to exposure to certain chemicals due to the immaturity of their physiological functions. Infants are more vulnerable due to the lack of enzymes to break down and remove toxins, and particularly sensitive to harmful substances that can affect endocrine, immune or nervous systems [8,9].

Fragrance allergens, preservatives, plasticizers, and synthetic musks are common ingredients in personal care products. Fragrances provide nice and attractive scents to make the product more attractive. Preservatives are essential to deliver a safe product to consumers; they are intended to actively prevent microbial growth within cosmetic products. In the case of baby wipes, the wet tissue liquids are aqueous; the storage temperature and the hard surface wipe (commonly cellulose) create an optimal medium for microbial growth. Plasticizers (phthalates and adipates) are mainly employed as fragrance solvents. Synthetic musks and plasticizers do not appear as such in the cosmetics label; musks are present under the terms “fragrance” or “parfum”. These families of chemicals are regulated in Europe by the Regulation (EC) No 1223/2009 and its subsequent amendments [10]. In this way, 26 fragrances must be monitorized, the so-called suspected allergen substances or fragrance allergens. Their presence must be indicated in the list of ingredients when their concentrations exceed 0.01% for rinse-off products, and 0.001% for leave-on products. One of these fragrances, lyral®, was recently proposed to be transferred from Annex III (list of substances allowed in cosmetics with restrictions) to Annex II (list of substances prohibited in cosmetic products).

Parabens (esters of p-hydroxybenzoic acid) are the preservatives most frequently used due to their broad antimicrobial spectrum and low cost. It is estimated that 75-90% of cosmetics contain parabens at levels between 0.01-0.3% [11]. Their maximum permitted concentration is 0.4% for a single ester and 0.8% for mixture of esters. Isopropyl-, isobutyl-, phenyl-, benzyl-, and pentylparaben have been recently banned for their use in cosmetics [1], and ethyl-, methyl-, propyl- and butylparaben are categorized as potential endocrine disrupters. In addition, propyl- and butylparaben have been banned in Denmark in products for children under 3 years [12]; they will be banned in all Europe form April 2015 for their use in the diaper area for children below 3 years, and their maximum permitted concentration in other cosmetic products will be 0.14% (instead 0.8%)[13]. Phenoxyethanol is one of the most commonly used preservatives and its maximum permitted concentration is 1%. The France National Agency of Security of Medicaments (ANSM) has

recently proposed to avoid the use of phenoxyethanol in products intended for children under 3 years, and to reduce the maximum permitted concentration to 0.4% in other personal care products [4,14]. Maximum permitted concentration for triclosan, was recently decreased to 0.2-0.3% in several products. 3-Iodo-2-propynyl butylcarbamate (IPBC) is banned in products for children under 3 years, except in bath products, shower gels and shampoos (0.02%); for leave-on products its maximum permitted concentration is 0.01%. The bromine-containing preservative bronidox can also be present in personal care products at a maximum concentration of 0.1%. The antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) can be used without restrictions.

Concerning synthetic musks and phthalates, European legislation on cosmetics banned the use of musk ambrette, moskene and tibetene since 1995 due to their bioaccumulative properties. Other musks (phantolide, tonalide and ketone) are allowed with restrictions. Diethyl phthalate (DEP) is widely used in personal care products; however, the European Commission on Endocrine Disruption has listed DEP as a priority substance. Other six phthalates: dibutyl phtalate (DBP), dimethoxyethyl phthalate (DMEP), diisopentyl phthalate (DIPP), dipentyl phthalate (DPP), benzyl butyl phthalate (BBP) and di(2-ethylhexyl)phthalate (DEHP) have been linked to endocrine disruption and they are forbidden in cosmetics and personal care products.

In last years, some efforts have been made to determine all these target families of cosmetics ingredients, and several analytical methods based in traditionally procedures (liquid-liquid, and solid-liquid extractions), and current sample preparation (stir-bar sorptive extraction, matrix-solid-phase dispersion, or pressurized liquid extraction, among others) have been proposed [14-19]. Nevertheless, there is a lack of studies devoted to the simultaneous determination of several families of cosmetic ingredients, and there are not specific analytical studies for this particular cosmetic product: the baby wipes. Most literature only includes aspects related with allergies [20-22] and only two studies include the determination of a reduced number of parabens [23,24]. Nevertheless, the analytical control of this product is essential to guarantee product safety for babies and children. Pressurized liquid extraction (PLE) is a very suitable technique to extract cosmetic ingredients, being analytes efficiently extracted from the samples at high pressure, minimizing sample preparation time [19,25,26].

The aim of this study is the development of a PLE and gas chromatography-mass spectrometry (GC-MS) method for the rapid (25 minutes) simultaneous determination of sixty-five chemicals belonging to four families of cosmetic ingredients in baby wipes and wet toilet paper.

2. EXPERIMENTAL

2.1. Chemicals, materials and samples

The studied compounds, their purity, suppliers, CAS numbers, retention time and quantification and identification ions are summarized in **Table 1**. Also, European legislation restrictions are included. Deuterated methyl-4-hydroxybenzoate-2,3,5,6-d_4 (MeP_d_4; 98atom% D), benzyl_d_7 alcohol (98atom% D) and bis(2-ethylhexyl)phthalate-3,4,5,6-d_4 (DEHP_d4; 98atom% D) used as surrogate standard, were obtained from C/D/N Isotopes (Quebec, Canada), Aldrich (St. Louis, MO, USA), and Fluka Chemie GmbH (Steinheim, Germany), respectively. Acetone, ethyl acetate, n-hexane, methanol and acetonitrile were provided by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Sand (200-300 µm mesh particle size) was provided by Scharlau (Barcelona, Spain). Florisil (60-100 mesh) was purchased from Supelco Analytical (Bellefonte, PA, USA) and anhydrous sodium sulphate (99 %) from Panreac (Barcelona, Spain). Individual stock solutions were prepared in acetone, isooctane or methanol. Further dilutions and mixtures were prepared in acetone or ethyl acetate. All solutions were stored in amber glass vials at -20°C. All solvents and reagents were of analytical grade.

Metallic, and glass material; sorbents (Florisil and sodium sulphate anhydrous) were baked at 230°C for 12 hours before use to eliminate possible phthalate contamination. All materials were allowed to cool down wrapped with aluminum foil and Florisil and sodium sulphate anhydrous in desiccator.

Table 1. Target compounds: chemical names, suppliers, purity, CAS, and EU restrictions (EC No 1223/2009)

Fragrance allergens[a]	***Purity (%)***	***CAS***	**Retention time (min)**	**Quantification** and identification ions	***Maximum concentration permitted*** **[10]**
Pinene	≥99 [b]	80-56-8	5.18	77 (27), **93 (100)**, 121 (13)	n.r
Limonene	97[b]	5989-27-5	6.82	**68 (100)**, 93 (76),121 (25)	n.r
Benzyl alcohol	99[b]	100-51-6	6.86	77 (73), 79 (115), **108 (100)**	1% (as preservative)
Linalool	97[b]	78-70-6	7.74	**71 (100)** ,93 (84) ,121 (24)	n.r
Methyl-2-octynoate	≥99[b]	111-12-6	8.85	79 (66) ,**95 (100)** ,123 (73)	n.r
Citronellol	95[b]	106-22-9	9.10	**69 (100)** ,95 (49) ,109 (18)	n.r
Citral	95[b]	5392-40-5	9.25/9.48	**69 (100),** 94 (17), 109 (10)	n.r
Geraniol	≥96[b]	106-24-1	9.33	**69 (100)**, 93 (18) ,111 (6)	n.r
Cinnamal	≥93[b]	104-55-2	9.54	77 (35), 103 (50), **131 (100)**	n.r
Hydroxycitronellal	≥95[b]	107-75-5	9.60	**59 (100)**, 71 (13) , 81 (43)	1%
Anise alcohol	98[b]	105-13-5	9.62	109 (77), 121 (55), **138 (100)**	n.r
Cinnamyl alcohol	98[b]	104-54-1	9.80	**92 (100)**, 105 (53), 115 (54)	n.r
Eugenol	99[b]	97-53-0	10.18	103 (28),131 (27),**164 (100)**	n.r
Methyleugenol	99[b]	93-15-2	10.45	147 (31), 163 (29), **178 (100)**	0.01% (fine fragrance); 0.004% (eau de toilette) ; 0.002% (fragrance cream); 0.0002% (other leave-on products); 0.001% (rinse-off products)
Isoeugenol	98[b]	97-54-1	10.53/10.79	103(22), 131 (20), 164 (100)	0.02%
Coumarin	99[b]	91-64-5	10.79	90 (42), 118 (110), **146 (100)**	n.r
α-isomethyl ionone	≥85[b]	127-51-5	11.02	107 (58), **135 (100)**, 150 (61)	n.r
Lilial®	≥85[b]	80-54-6	11.33	131 (39), 147 (40), **189 (100)**	n.r
Amyl cinnamal	97[b]	122-40-7	12.28	115 (89), **129 (100)**, 145 (57)	n.r
Lyral®[g]	≥97[b]	31906-04-4	12.43	79 (74), 93 (78), **136 (100)**	n.r
Amylcinnamyl alcohol	≥85[b]	101-85-9	12.61	91(88), 115 (60), **133 (100)**	n.r
Farnesol	95[b]	4602-84-0	12.66/12.90	**69 (100)**, 93 (27), 107 (15)	n.r
Hexylcinnamal	≥95[b]	101-86-0	13.27	**129 (100)**, 145 (51), 216 (40)	n.r
Benzyl benzoate	98[b]	120-51-4	13.54	77 (28), 91 (47), **105 (100)**	n.r
Benzyl salicylate	≥99[b]	118-58-1	15.04	65 (11), **91 (100)**, 228 (12)	n.r
Benzyl cinnamate	99[b]	103-41-3	18.56	**91 (100)**, 131 (90), 192 (63)	n.r
Preservatives	***Purity (%)***	***CAS***	**Retention time (min)**	**Quantification** and identification ions	***Maximum concentration permitted*** **[5]**
Bronidox	≥99[c]	30007-47-7	9.00	85 (27), 106 (49), **137 (100)**	0.1%
Phenoxyethanol (PhEtOH)	99[c]	122-99-6	9.06	77 (28), **94 (100)**, 138 (31)	1%
Methyl paraben (MeP)	99[b]	99-76-3	10.76	93 (21), **121 (100)**, 153 (35)	0.4% as acid (single); 0.8% as acid (mixtures)
Butylated hydroxyanisole	98.5[c]	25013-16-5	11.00	137 (63), **165 (100)**, 180 (51)	n.r
Butylatedhydroxytoluene	99[c]	128-37-0	11.22	177 (8), **205 (100)**, 220 (25)	n.r
Ethyl paraben (EtP)	99[b]	120-47-8	11.22	**121 (100)**, 138 (21), 166 (18)	0.4% as acid (single); 0.8% as acid (mixtures)
Isopropyl paraben (iPrP)	≥99[b]	4191-73-5	11.43	**121 (100)**, 138 (39), 180 (14)	Prohibited
Propyl paraben (PrP)[h]	99[b]	94-13-3	11.96	**121 (100)**, 138 (58), 180 (7)	0.14% as acid (single); 0.8% as acid (mixtures) Prohibited in leave-on cosmetics designed for application on the nappy area of children under 3 years.
3-Iodo-2-propynyl butylcarbamate (IPBC)	97[c]	55406-53-6	12.32	100 (15), **165 (100)**, 182 (50)	Rinse-off: 0.02% (not to be used in products for children under 3 years, except in bath products); leave-on: 0.01%; deodorants: 0.0075%
Isobutyl paraben (iBuP)	≥97[b]	4247-02-3	12.46	93 (12), **121 (100)**, 138 (58)	Prohibited
Butyl paraben (BuP)[h]	99[b]	94-26-8	12.90	**121 (100)**, 138 (84), 194 (6)	0.14% as acid (single); 0.8% as acid (mixtures) Prohibited in leave-on cosmetics designed for application on the nappy area of children under 3 years.
Triclosan	≥97[c]	3380-34-5	18.75	218 (93), **288 (100)**, 289 (93)	0.3%
Benzyl paraben (BzP)	99[b]	94-18-8	18.95	91 (46), **121 (100)**, 228 (21)	Prohibited

Table 1. Continuation

Phthalates	***Purity (%)***	***CAS***	**Retention time (min)**	**Quantification** and identification ions	***Maximum concentration permitted* [5]**
Dimethyl phthalate (DMP)	98[c]	131-11-3	10.81	77 (13), 194 (66), **163** (100)	n.r
Diethyl phthalate (DEP)	98[b]	84-66-2	11.79	**149** (100), 150 (12), 177 (24)	n.r
Diisobutyl phthalate (DIBP)	99[f]	84-69-5	14.79	57 (12), **149** (100), 223 (6.8)	n.r
Dibutyl phtalate (DBP)	99[b]	84-74-2	16.42	**149** (100), 150 (9), 223 (4.9)	Prohibited
Dimethoxyethyl phthalate (DMEP)	94[f]	117-82-8	17.07	**59** (100), 104 (18), 149 (29)	Prohibited
Diisopentyl phthalate (DIPP)	99.5[b]	605-50-5	18.20	71 (23), **149** (100), 237 (10)	Prohibited
Dipentyl phthalate (DPP)	99.2[b]	131-18-0	19.09	71 (16), **149** (100), 237 (5.6)	Prohibited
Benzyl butyl phthalate (BBP)	98[b]	85-68-7	20.47	91 (53), **149** (100), 206 (24)	Prohibited
Diisoheptyl phthalate (DIHP)	99[b]	71888-89-6	21.05	**149** (100), 223 (7), 265 (100)	n.r
Di(2-ethylhexyl)phthalate (DEHP)	99.5[c]	117-81-7	21.51	167 (30), **149** (100), 279 (10)	Prohibited
Diciclohexyl phthalate (DCHP)	99[b]	84-61-7	21.46	55 (19), **149** (100), 167 (31)	n.r
Diphenyl phthalate (DPhP)	98[b]	84-62-8	21.63	77 (19), 153 (4), **225** (100)	n.r
Di-n-octyl phthalate (DOP)	≥98[d]	117-84-0	22.68	**149** (100), 223 (22), 279 (6.2)	n.r
Diisononyl phthalate (DINP)	99[b]	28553-12-0	23.43	**149** (100), 279 (7), 293 (17)	n.r
Diisodecyl phthalate (DIDP)	99[b]	26761-40-0	24.30	71 (34), **149** (100), 307 (20)	n.r
Musks	***Purity (%)***	***CAS***	**Retention time (min)**	**Quantification** and identification ions	***Maximum concentration permitted* [5]**
Cashmeran	≥95[f]	33704-61-9	11.23	135 (43), **191** (100), 206 (57)	n.r
Celestolide	98[f]	13171-00-1	12.99	173 (22), **229** (100), 244 (44)	n.r
Phantolide	≥98[e]	15323-35-0	13.50	187 (11), **229** (100), 244 (24)	n.r
Musk Ambrette	99[b]	83-66-9	14.49	**253** (100), 254 (13), 268 (35)	Prohibited
Traseolide	99[e]	68140-48-7	14.70	43 (41), **215** (100), 258 (14)	n.r
Galaxolide	55.8[b]	1222-05-5	14.79	213 (23), **243** (100), 258 (20)	n.r
Tonalide	98[f]	1506-02-1	14.95	43 (48), **243** (100), 258 (26)	Leave-on products: 0.1% (except hydroalcoholic products:1%; fine fragrance:2.5% and fragrance cream:0.5%); rinse -off products: 0.2%
Musk Moskene	≥99[b]	116-66-5	13.35	**263** (100), 264 (20), 278 (8.9)	Prohibited
Musk Tibetene	≥99[b]	145-39-1	16.29	43 (33), **251** (100), 266 (28)	Prohibited
Ambrettolide	≥97[b]	7779-50-2	16.18	**67** (100), 81 (98), 96 (89)	n.r
Musk Ketone	≥98[b]	81-14-1	16.95	191 (24), 294 (26), **279** (100)	Fine fragrance:1.4%; eau de toilette: 0.56%; other products:0.042%

[a] The presence of the substance must be indicated in the list of ingredients when its concentration exceeds 0.001% (leave-on products) and 0.01% (rinse-off products). [b]Sigma Aldrich Chemie GmbH (Steimheim, Germany). [c]Fluka Chemie GmbH (Steimheim, Germany). [d]Supelco Analytical (Bellefonte, USA). [e]LGC Standards GmbH (Wesel, Germany). [f]Dr Ehrenstorfer (Ausburg, Germany). [g]Is proposed to be excluded completely from cosmetics and personal care products. n.r: no restricted by EC No 1223/2009. [h] The legislation requirements will enter into force from April 2015.

Baby wipes and wet toilet paper samples intended for children under 3 years of age from national and international brands were obtained from local sources. The samples were kept in their original containers at room temperature, until their analysis.

2.2. PLE procedure

Extractions were performed on an ASE 150 (Dionex, Co., Sunyvale, CA, USA), equipped with 10 mL stainless steel cells and 60 mL collection vials. Two cellulose filters (Dionex, 27 mm) were placed at each end of the PLE cell and also, in the bottom, a polyethylene frit (Supelco, 12 mL) was employed at the bottom. An individual baby wipe was weighted (wipes weight range: 2.48-8.03 g), introduced into the cell and spiked with 10 µL of the surrogate solution (50 µg mL^{-1}). Previously, 1.5 g of clean sand were placed (different sorbents were tested during the method optimization) in the cell. For the preparation of fortified samples, the baby wipe was spiked with 100 µL of the corresponding acetone solution of the target compounds to get the desired final concentration. Finally, the dead volume was immediately filled with sand to avoid losses of the most volatile analytes. The cell was tightly closed and placed into the PLE system. The extraction pressure was 1500 psi, the flush volume was 60%, and the purge time 60 s. Different extraction conditions were tested along the study. Methanol, acetonitrile, ethyl acetate, acetone, and the mixture hexane:acetone (1:1, v/v) were employed as extraction solvent. Three temperatures were studied: 70, 90 and 110°C, as well as three different times 5, 10 and 15 min. The optimal extraction conditions were: MeOH, 110°C, and 5 min. In all cases, the extracts were levelled to a final volume of 20 mL with the extraction solvent. Then, PLE extracts were diluted in ethyl acetate (1:5, v/v), filtered through a 0.20 µm PTFE filters, and directly analyzed by GC-MS, without any pre-concentration or clean-up additional steps.

2.3. GC-MS analysis

The GC–MS analysis was performed using an Agilent 7890A (GC)-Agilent 5975C inert MSD with triple axis detector and an Agilent 7693 autosampler from Agilent Technologies (Palo Alto, CA, USA). The temperatures of the transfer line, the quadrupole and the ion source were set at 290, 150 and 230°C, respectively. The system was operated by Agilent MSD ChemStation E.02.00.493 software.
Separation was carried out on a SLB^{TM}-5ms capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness) obtained from Supelco Analytical (Bellefonte, PA, USA). Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 mL min^{-1}. The GC oven temperature was programmed from 60°C (held 1 min) to 100°C at 8°C min^{-1}, to 150°C at 20°C min^{-1}, to 200°C at 25°C min^{-1} to 220°C at 8°C min^{-1} and 30°C min^{-1} to 290°C (held 10 min). After 1 min, the split valve was opened (75 mL min^{-1}), and the injector temperature was kept at 260°C. The injection volume was 1 µL. The electron multiplier was set at a nominal value of 1376 V.

2.4. Statistical analysis

Basic and descriptive statistics, as well as experimental design analysis, were performed using Statgraphics-Plus v5.1 (Manugistics, Rockville, MD, USA) as software package. Experimental design was applied in the optimization of the extraction method to analyze the simultaneous effect of the experimental parameters affecting PLE.

3. RESULTS AND DISCUSSION

The chromatographic conditions were optimized to achieve an efficient separation of the target compounds: 26 fragrance allergens, 13 preservatives, 15 phthalates, and 11 musks (see conditions in the experimental section). For GC-MS analysis, the mass spectra detector (MSD) was operated in selected ion monitoring (SIM) mode, monitoring three ions per compound (**Table 1**).

3.1. Optimization of the extraction process

The influence of the main variables potentially affecting the PLE procedure must be evaluated to obtain an efficient extraction. In this way, the study of the influence of PLE conditions was accomplished in several steps. First, the sorbent added to the extraction cell, and the extraction solvent were evaluated with the intention of obtaining homogeneous and clean extracts as well as high chromatography response. Once the sorbent was selected, the solvent and other variables, such as the temperature and the extraction time, were optimised by means of a multifactor experimental design.

3.1.1. Preliminary tests

Sorbent screening

Different sorbents were tested during the PLE procedure. The effect of sand (A), and mixtures of sand with Florisil (1:1, w/w) (B), and sand with Florisil and anhydrous sodium sulphate (1:1:1, w/w) (C), were compared. Also, the extraction without the inclusion of a sorbent agent in the extraction cell (D) was tested. Two cellulose filters and a polyethylene frit were placed at the cell bottom followed by 0.5 g of sand, 1 g of the corresponding sorbent or mixture of sorbents, and the baby wipe; finally, the cell was completely filled with sand and other cellulose filter was placed on the top. In the case D, a baby wipe was placed into the extraction cell on top of the polyethylene frit. All extractions were performed with MeOH at 90°C for 10 minutes. In this study, a real non-spiked baby wipe sample containing 14 target compounds was employed, in this way the real analyte-matrix interactions are considered. A one-way analysis of variance (ANOVA) was carried out and the results are summarized in **Table 2**. The sorbent was statistically significant for limonene, benzyl alcohol, DIBP and PrP ($p<0.05$). As can be seen in the mean-value charts showed for limonene and DIBP (**Figure 1**), the lowest response was obtained by not using sorbent (D), whereas responses were similar employing only sand (A) or the sorbents mixtures: (B), (C). Besides, turbid extracts were obtained when sorbents mixtures were used; therefore, we decided to use sand as sorbent since it offers the cleanest extracts with good extraction response.

Table 2. F ratios and p values obtained in the ANOVA study of the sorbents[a]

Compounds	F	P
Limonene	**27**	**0.011**
Benzyl alcohol	**106**	**0.0015**
α-isomethylionone	5.0	0.11
Lilial®	3.5	0.16
Hexylcinnamal	0.71	0.61
DEP	4.1	0.14
DIBP	**28**	**0.010**
PhEtOH	0.76	0.59
MeP	2.2	0.26
BHT	1.1	0.47
EtP	2.4	0.24
PrP	**11**	**0.041**
BuP	0.18	0.90
Galaxolide	1.3	0.42

[a] p<0.05 means statistical significance

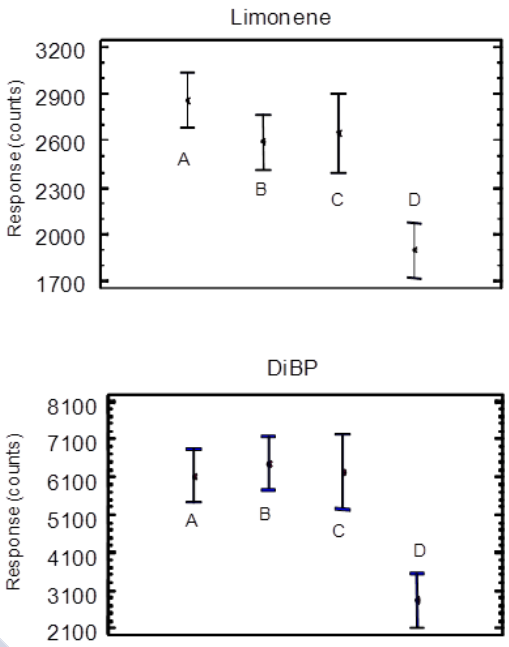


Fig.1. ANOVA mean charts for the sorbent

Solvent screening

Acetonitrile (ACN), methanol (MeOH), ethyl acetate (EtAc), and hexane:acetone (1:1, v/v) were tested. In this case, a real non-spiked baby wipe sample containing 13 target compounds of the four cosmetics families of additives was employed. Extraction conditions were 90°C and 10 minutes, taking into account previous results, and the sorbent employed was sand. As can be seen in **Figure 2**, MeOH provides the highest responses for the majority of the compounds. When EtAc and the mixture hexane:acetone (1:1, v/v) were used, the formation of two phases and foams were observed and, therefore, these solvents were discarded. MeOH and ACN were included in the next study, and also acetone was incorporated due to its similar chemical properties with the other preselected solvents.

3.1.2. Experimental design

Once the sorbent was selected (sand) and two solvents (MeOH and ACN) were pre-selected, other two parameters which can drastically affect extraction, PLE temperature and time, were optimized. All the optimization studies have been developed employing non-spiked real samples. In this way, the real matrix-analyte interactions are considered in the selection of the most suitable extraction conditions. A real non-spiked baby wipe sample containing 15 target compounds (limonene, benzyl alcohol, α-isomethylionone, lilial®, hexylcinnamaldehyde, DEP, DEHP, DIBP, phEtOH, MeP, BHT, EtP, PrP, BuP, and galaxolide) was employed. The extraction process was optimized by means of a multifactor experimental design $3*2^2$ and three factors were analyzed: elution solvent (A), extraction temperature (B), and extraction time (C) (**Table 3**).

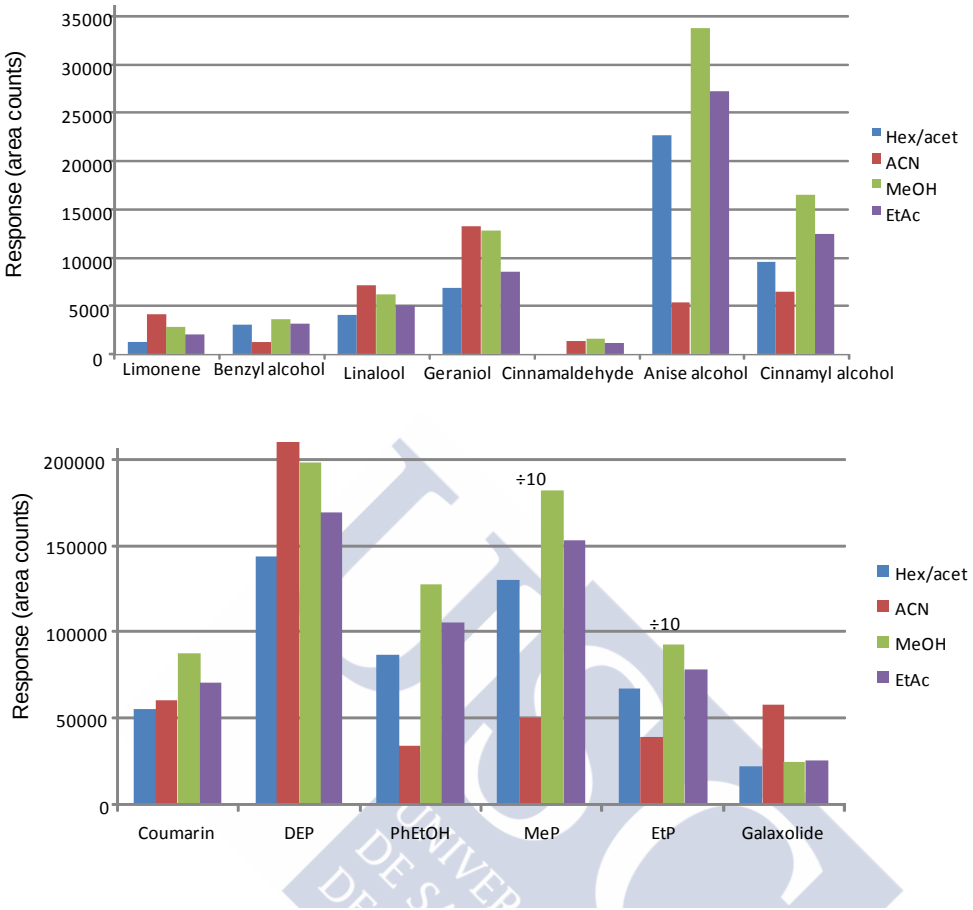


Fig.2. Comparative responses for different elution solvents (n=2).

Table 3. Factors and levels considered in the experimental design.

Factor	*Key*	*Low level (-)*	*Central level*	*High level (+)*
Solvent	A	Methanol	Acetonitrile	Acetone
Temperature (ºC)	B	70	90	110
Time (min)	C	5	10	15

The efficiency of three solvents (MeOH, ACN, and acetone) was tested. MeOH and ACN were chosen because in preliminary studies showed cleaner extracts and the best extraction efficiency, and acetone was included in the experimental design because it has similar chemical characteristics and it also provides clean extracts. Other two parameters which considerably can affect PLE, temperature (B) and time (C), were studied: 70 and 110°C (temperature) and 5 and 15 minutes (extraction time). These levels were chosen based on previous studies [19,25,26]. Also, two intermediate levels 90°C and 10 minutes were included**.**

The results for the experimental design are shown in the ANOVA table (**Table 4**). As can be seen, the temperature was the most relevant factor being statistically significant or very close to this level for several analytes (α-isomethylionone, lilial®, DIBP, BHT, and galaxolide). Solvent (A) was significant for DEHP. The interactions solvent-temperature (AB) and solvent-time (AC) were not significant in any case, except for galaxolide, and DIBP, respectively. Solvent-solvent (AA) interaction was only

significant for these last compounds. Extraction time (C) and its interaction with temperature (BC) were not significant ($p>0.05$) in any case, therefore BC is not shown in the Pareto charts, and interaction plots (**Figures 3 and 4**).

Table 4. F ratios and p values obtained in the analysis of variance for the baby wipe study[a]

	A Solvent		B Temperature		C Time		AA		AB		AC	
Compounds	**F**	**P**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Limonene	0.74	0.42	0.39	0.55	0.26	0.63	2.9	0.13	1.1	0.32	3.1	0.12
Benzyl alcohol	0.40	0.55	0.38	0.56	0.06	0.82	1.3	0.29	1.1	0.32	0.91	0.37
α-isomethylionone	0.00	0.98	**5.8**	**0.04**	0.14	0.72	2.4	0.16	0.35	0.57	0.15	0.71
Lilial®	0.47	0.51	**6.1**	**0.04**	0.19	0.68	4.0	0.08	4.0	0.09	1.6	0.25
Hexylcinnamal	1.6	0.25	2.6	0.16	0.19	0.68	4.2	0.08	2.2	0.19	3.9	0.09
DEP	0.01	0.92	2.2	0.19	0.04	0.85	3.6	0.11	2.0	0.20	1.3	0.29
DEHP	**5.9**	**0.04**	0.57	0.46	0.13	0.73	0.83	0.39	0.20	0.66	0.18	0.69
DIBP	1.3	0.30	**11**	**0.01**	0.16	0.70	**11**	**0.01**	0.62	0.46	**13**	**0.01**
PhEtOH	0.42	0.54	0.32	0.59	0.07	0.80	0.95	0.36	1.1	0.32	0.94	0.37
MeP	0.08	0.79	0.54	0.49	0.06	0.82	1.5	0.26	1.4	0.28	1.2	0.30
BHT	0.00	0.96	**5.8**	**0.04**	0.00	0.96	1.6	0.24	1.9	0.20	1.3	0.28
EtP	0.00	0.99	1.2	0.32	0.00	0.96	1.5	0.27	1.8	0.23	1.8	0.22
PrP	0.09	0.78	1.9	0.21	0.09	0.78	2.8	0.14	2.6	0.15	2.5	0.16
BuP	0.38	0.56	3.0	0.12	0.06	0.81	3.6	0.10	3.6	0.10	2.9	0.13
Galaxolide	0.69	0.43	**12**	**0.01**	0.04	0.84	**6.8**	**0.04**	**6.3**	**0.04**	2.4	0.16

In the Pareto charts, the standardized effects are plotted in decreasing order of absolute magnitude, thus making it easier to see which ones are the most important factors and interactions. In addition, the line drawn on the chart indicates whether an effect is statistically significant at a specified significance level (in this case, 95%). Main effect plots show how the response varies when each factor is changed from its low level to its high level, while all other factors are held at the center of the experimental domain. **Figure 3** shows the Pareto charts and main effect plots for some representative analytes of the different studied families (lilial®, BHT, galaxolide, and DIBP). As can be seen, temperature (factor B) exceeds the significance limit for these compounds; the main effect plots (**Figure 4a and 4b**) show higher response when extractions are carried out at 110ºC (+) and employing MeOH (-) as extraction solvent. **Figure 4b and 4c** shows interactions solvent-temperature (AB) and solvent-time (AC) for galaxolide and DIBP, respectively. In the first case, higher responses were obtained employing MeOH at 110ºC, for DIBP, higher efficiency was obtained using MeOH with 5 minutes of extraction time (these trends were also observed for most compounds). Therefore, in view of the results, the selected conditions involve the PLE extraction at 110ºC for 5 minutes using MeOH as extraction solvent.

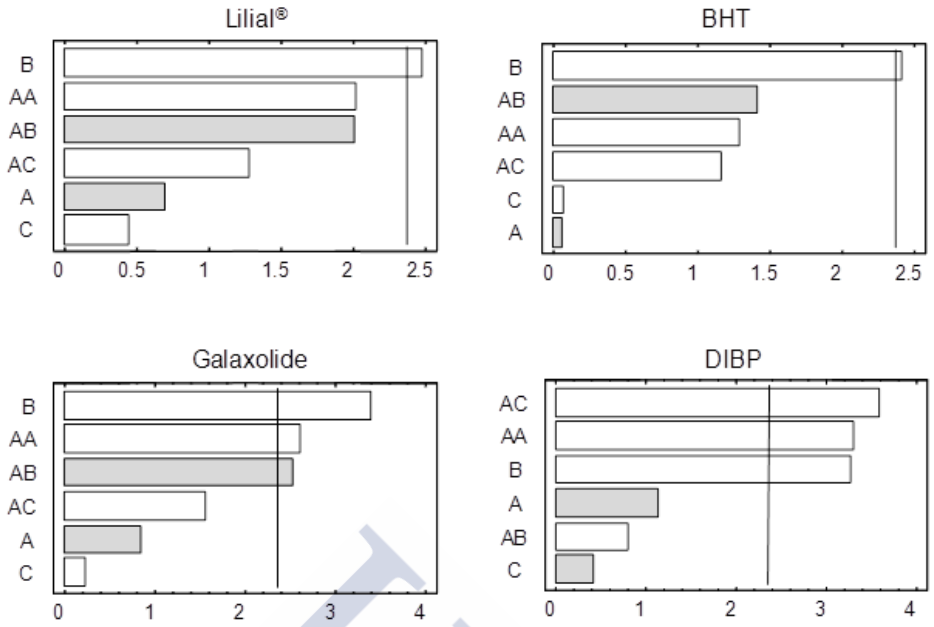


Fig.3. Pareto charts for lilial®, BHT, galaxolide and DIBP.

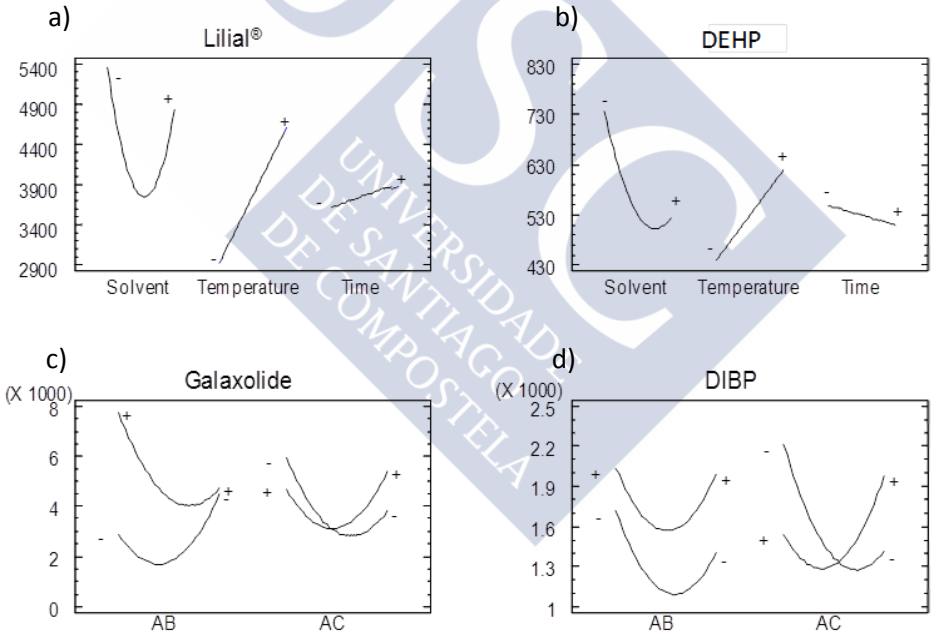


Fig.4. Main effect plots for (a) lilial® (b) DEHP, and interaction plots for (c) galaxolide, (d) DIBP.

3.2. Method performance

The GC-MS method performance parameters were studied for all the target compounds and they are summarized in **Table 5**. Regarding the instrumental linearity, calibration standards were prepared in ethyl acetate covering a concentration range from 2 to 2000 ng mL^{-1} (see specific ranges in **Table 5**). The method exhibited a direct proportional relationship between the amount of each analyte and the chromatographic response. Correlation coefficients R≥0.991 were obtained in all cases. Instrumental method precision was studied within a day (n=5) and among days (n=8) at 100

ng mL^{-1} (other concentration levels, 250 and 500 ng mL^{-1} were also evaluated, data not shown). Relative standard deviation (RSD) values were in all cases below 10% and below 15% for intra-day precision and inter-day precision, respectively. Instrumental detection limits (IDLs) were calculated as the concentration giving a signal-to-noise ratio of three (S/N=3). IDLs were in the low ng mL^{-1} for most compounds. For fragrance allergens and preservatives IDLs were below 6 ng mL^{-1} (excluding farnesol, and IPBC, 70 and 10 ng mL^{-1}, respectively). For phthalates, they were below 1 ng mL^{-1}, excluding DIHP, DINP, and DIDP, which are mixtures of isomers, and their chromatographic signal is composed of several analytical peaks. In the case of musks, their IDLs were in the low ng mL^{-1} with values between 0.16-3.0 ng mL^{-1}.

Table 5. Instrumental quality parameters (GC-MS).

Fragrance allergens	**Lineal Range (ng mL^{-1})**	**Correlation coefficient (R)**	**IDL (ng mL^{-1})**	**Intra-day precision[a] (RSD, %)**	**Inter-day precision[a] (RSD, %)**
Pinene	2-1000	0.9998	0.22	0.18	6.4
Limonene	2-1000	0.9996	0.41	0.24	1.2
Benzyl Alcohol	5-1000	0.9981	1.2	1.3	0.30
Linalool	5-1000	0.9974	1.5	0.87	2.5
Methyl-2-octynoate	5-1000	0.9970	1.5	1.4	2.8
Citronellol	10-1000	0.9967	2.1	0.28	1.9
Citral	10-1000	0.9969	2.8	0.34	2.3
Geraniol	10-1000	0.9961	2.8	6.3	2.3
Cinnamal	10-1000	0.9988	2.2	0.39	5.0
Hydroxycitronellal	5-1000	0.9952	1.3	0.74	3.0
Anise Alcohol	20-1000	0.9978	6.1	1.3	1.8
Cinnamyl Alcohol	20-1000	0.9982	4.4	2.6	3.2
Eugenol	5-1000	0.9973	1.2	1.3	2.6
Methyleugenol	5-1000	0.9995	1.0	5.2	5.5
Isoeugenol	10-1000	0.9993	2.6	1.1	7.0
Coumarin	10-1000	0.9991	2.2	1.2	14
α-isomethyl ionone	2-1000	0.9983	0.61	0.27	13
Lilial®	2-1000	0.9978	0.44	0.35	5.4
Amyl Cinnamal	20-1000	0.9960	3.2	0.77	12
Lyral®	10-1000	0.9960	3.0	0.01	1.9
Amylcinnamyl Alcohol	20-1000	0.9990	6.0	1.3	3.1
Farnesol	250-1000	0.9988	70	0.20	8.3
Hexylcinnamal	10-1000	0.9979	3.0	0.87	6.4
Benzyl Benzoate	10-1000	0.9990	1.9	0.42	3.2
Benzyl Salicylate	10-1000	0.9951	2.3	1.3	2.2
Benzyl Cinnamate	10-1000	0.9961	2.3	1.8	3.2

Table 5. Continuation

Preservatives	**Lineal Range (ng mL^{-1})**	**Correlation coefficient (R)**	**IDL (ng mL^{-1})**	**Intra-day precision[a] (RSD, %)**	**Inter-day precision[a] (RSD, %)**
Bronidox	10-1000	0.9971	2.6	1.6	2.8
PhEtOH	5-1000	0.9954	1.1	2.5	2.4
MeP	10-1000	0.9934	3.0	4.9	13
BHA	2-1000	0.9910	0.50	6.0	10
BHT	2-1000	0.9954	0.15	1.3	5.6
EtP	10-1000	0.9954	3.2	3.1	10
iPrP	20-1000	0.9971	3.2	0.24	15
PrP	20-1000	0.9942	3.1	1.7	9.7
IPBC	50-1000	0.9918	10	7.9	8.9
iBuP	20-1000	0.9936	3.9	0.88	11
BuP	10-1000	0.9914	2.8	2.0	9.8
Triclosan	20-1000	0.9934	3.2	2.1	14
BzP	20-1000	0.9965	6.2	5.9	15
Phthalates	**Lineal Range (ng mL^{-1})**	**Correlation coefficient (R)**	**IDL (ng mL^{-1})**	**Intra-day precision[a] (RSD, %)**	**Inter-day precision[a] (RSD, %)**
DMP	2-1000	0.9995	0.26	0.06	4.0
DEP	2-1000	0.9995	0.13	0.14	4.7
DIBP	2-1000	0.9966	0.18	0.03	3.6
DBP	2-1000	0.9975	0.44	0.28	4.5
DMEP	2-1000	0.9946	0.59	0.37	9.6
DIPP	2-1000	0.9941	0.66	0.18	3.6
DPP	2-1000	0.9977	0.37	0.29	5.0
BBP	5-1000	1.0000	0.88	0.07	5.2
DIHP	100-2000	0.9970	20	10	15
DEHP	2-1000	0.9957	0.36	0.20	12
DCHP	2-1000	0.9931	0.22	1.6	4.9
DPhP	2-1000	0.9952	0.51	0.60	7.8
DNOP	2-1000	0.9965	0.48	0.69	3.9
DINP	100-2000	0.9992	20	10	13
DIDP	200-2000	0.9998	40	6.4	14
Musks	**Lineal Range (ng mL^{-1})**	**Correlation coefficient (R)**	**IDL (ng mL^{-1})**	**Intra-day precision[a] (RSD, %)**	**Inter-day precision[a] (RSD, %)**
Cashmeran	2-1000	0.9969	0.53	0.96	4.1
Celestolide	2-1000	0.9946	0.16	0.17	4.6
Phantolide	2-1000	0.9957	0.16	0.02	3.7
Musk Ambrette	5-1000	0.9903	1.5	0.92	5.9
Traseolide	2-1000	0.9901	0.16	0.54	4.6
Galaxolide	2-1000	0.9954	0.22	0.32	3.3
Tonalide	2-1000	0.9968	0.21	0.55	3.7
Musk Moskene	2-1000	0.9925	0.61	1.0	5.3
Musk Tibetene	2-1000	0.9927	1.5	0.61	5.1
Ambrettolide	10-1000	0.9959	2.0	0.60	3.7
Musk Ketone	10-1000	0.9930	3.0	1.2	5.9

[a] For 100 ng mL^{-1} (excepted DIHP, DINP, DIDP: 200 ng mL^{-1}).

Method quality parameters for the whole procedure PLE/GC-MS analysis are shown in **Table 6**, and they were also evaluated using real baby wipe, and wet toilet paper samples. Recovery studies were carried out by applying the optimized PLE method to the extraction of 2 real spiked samples at 10 and 100 μg/wipe. Previous analyses of the samples showed the presence of some of the target compounds, and these initial concentrations were taken into account to calculate the recoveries. Recoveries were higher than 90 % for the majority of the studied compounds. These recoveries can be considered quantitative and no matrix effects were observed; therefore, quantification by external calibration can be effectively employed. The absence of matrix effect could be attributed to the fact that the proposed method provides clean extracts, and it does not require concentration (a baby wipe is extracted with 20 mL of solvent and the extract is further diluted 1:5, v/v). Precision was also evaluated (see RSD values in the table) attaining RSD values generally lower than 10 %. Therefore, the method can be considered suitable for the determination of all target fragrance allergens, preservatives, musks, and phthalates in real samples. Limits of detection and quantification (LODs and LOQs) were calculated as the compound concentration giving a signal-to-noise ratio of three (S/N=3) and ten (S/N=10), respectively using spiked samples at low level of concentration. The LOD values are shown in **Table 6** and they are expressed in μg g^{-1} and in μg per wipe. LOD for the fragrance allergens ranged from 0.0011 μg g^{-1} to 0.031 μg g^{-1} (0.0044-0.12 μg/wipe) (excluding farnesol). For preservatives, LOD values were between 0.00077-0.051 μg g^{-1} (0.0030-0.200 μg/wipe). For musks and phthalates these values were between 0.00067-0.015 μg g^{-1} (0.0026-0.0600 μg/wipe) (excluding DIHP, DINP and DIDP (0.40-0.80 μg/wipe). All the obtained LODs and LOQs were several orders of magnitude below the European legal requirements.

Table 6. Recoveries (%), precision (RSD,%), and LODs for the whole PLE/GC-MS method

Fragrance allergens	Recoveries (baby wipe)		Recoveries (wet toilet paper)		LOD	LOD
	10 μg/wipe	100 μg/wipe	10 μg/wipe	100 μg/wipe	(μg g^{-1})	(μg/wipe)
Pinene	80.2 (2.4)	92.3 (1.5)	84.0 (6.1)	88.6 (4.7)	0.0011	0.0044
Limonene	84.5 (1.9)	88.1 (0.02)	96.9 (2.2)	90.4 (0.15)	0.0021	0.0082
Benzyl Alcohol	100 (2.6)	115 (3.9)	106 (3.6)	107 (2.3)	0.0059	0.023
Linalool	93.6 (15)	105 (8.0)	82.7 (11)	95.8 (1.9)	0.0075	0.029
Methyl-2-octynoate	82.4 (2.2)	92.0 (4.8)	87.3 (15)	81.6 (0.81)	0.0077	0.030
Citronellol	104 (6.3)	107 (7.8)	104 (3.1)	96.6 (6.1)	0.011	0.043
Citral	93.3 (1.7)	81.2 (15)	92.4 (4.5)	97.5 (5.0)	0.014	0.056
Geraniol	89.4 (12)	81.7 (7.0)	101 (4.8)	85.8 (1.9)	0.015	0.057
Cinnamal	92.6 (1.6)	103 (1.8)	92.0 (2.5)	81.3 (1.6)	0.011	0.044
Hydroxycitronellal	86.3 (2.5)	115 (8.3)	82.9 (6.3)	93.6 (5.7)	0.0067	0.026
Anise Alcohol	91.0 (5.5)	115 (11)	96.9 (4.4)	100 (4.5)	0.031	0.12
Cinnamyl Alcohol	92.0 (0.40)	115 (11)	90.0 (5.8)	104 (5.5)	0.023	0.089
Eugenol	95.2 (1.6)	110 (4.1)	104 (4.2)	90.7 (2.6)	0.0063	0.025
Methyleugenol	86.3 (1.7)	95.8 (2.1)	84.6 (3.4)	80.4 (4.6)	0.0054	0.021
Isoeugenol	92.7 (3.6)	108 (1.5)	95.1 (3.9)	90.4 (3.1)	0.014	0.052
Coumarin	84.6 (2.5)	103 (0.80)	82.2 (3.0)	83.2 (0.61)	0.011	0.044
α-isomethyl ionone	99.3 (9.7)	98.1 (0.80)	89.6 (3.2)	80.1 (3.0)	0.0031	0.012
Lilial®	89.5 (2.2)	96.4 (1.4)	88.8 (3.5)	80.1 (1.0)	0.0023	0.0088
Amyl Cinnamal	86.5 (15)	99.5 (0.60)	107 (4.8)	92.1 (5.3)	0.016	0.064
Lyral®	94.9 (0.60)	113 (4.8)	103 (10)	104 (4.8)	0.015	0.060

Table 6. Continuation

Fragrance allergens	**Recoveries (baby wipe)**		**Recoveries (wet toilet paper)**		**LOD**	**LOQ**
	10 µg/wipe	**100 µg/wipe**	**10 µg/wipe**	**100 µg/wipe**	**(µg g^{-1})**	**(µg/wipe)**
Amylcinnamyl Alcohol	103 (1.3)	106 (3.2)	115 (1.9)	92.1 (5.3)	0.031	0.12
Farnesol	100 (12)	96.8 (6.5)	83.7 (1.2)	98.8 (3.5)	0.36	1.4
Hexylcinnamal	100 (4.1)	98.5 (1.2)	84.7 (5.7)	108 (3.4)	0.015	0.060
Benzyl Benzoate	94.3 (3.8)	96.1 (2.4)	85.6 (5.5)	104 (0.42)	0.0097	0.038
Benzyl Salicylate	99.5 (0.40)	119 (1.5)	115 (7.7)	109 (2.6)	0.012	0.047
Benzyl Cinnamate	112 (6.6)	104 (1.0)	95.8 (6.7)	83.4 (1.2)	0.012	0.046
Preservatives	**Recoveries (baby wipe)**		**Recoveries (wet toilet paper)**		**LOD**	**LOQ**
	10 µg/wipe	**100 µg/wipe**	**10 µg/wipe**	**100 µg/wipe**	**(µg g^{-1})**	**(µg/wipe)**
Bronidox	81.0 (2.5)	114 (5.6)	74.9 (15)	114 (4.8)	0.013	0.052
PhEtOH	---	---	---	---	0.0057	0.022
MeP	84.1 (3.1)	104 (13)	112 (3.9)	100 (4.8)	0.015	0.060
BHA	77.3 (1.0)	104 (15)	76.9 (2.9)	105 (3.7)	0.0026	0.010
BHT	78.4 (6.0)	99.0 (2.7)	75.0 (4.3)	111 (2.0)	0.00077	0.0030
EtP	84.3 (3.4)	103 (0.81)	95.5 (3.6)	94.0 (2.0)	0.017	0.065
iPrP	81.4 (2.8)	107 (4.1)	80.0 (4.6)	111 (0.70)	0.016	0.063
PrP	82.3 (2.7)	102 (3.3)	87.8 (4.5)	83.5 (6.6)	0.016	0.062
IPBC	82.3 (0.30)	109 (11)	82.2 (8.7)	84.8 (1.8)	0.051	0.20
iBuP	82.0 (2.8)	98.9 (2.5)	96.4 (4.9)	95.5 (3.2)	0.020	0.078
BuP	94.6 (1.3)	110 (6.1)	112 (15)	115 (2.6)	0.014	0.056
Triclosan	101 (0.10)	112 (6.6)	120 (4.6)	120 (3.5)	0.017	0.065
BzP	102 (1.0)	96.3 (2.9)	120 (4.1)	90.9 (8.5)	0.032	0.12
Plasticizers	**Recoveries (baby wipe)**		**Recoveries (wet toilet paper)**		**LOD**	**LOD**
	10 µg/wipe	**100 µg/wipe**	**10 µg/wipe**	**100 µg/wipe**	**(µg g^{-1})**	**(µg/wipe)**
DMP	89.2 (3.8)	93.3 (1.8)	113 (5.9)	101 (2.0)	0.0013	0.0052
DEP	72.3 (3.0)	92.4 (0.90)	80.2 (4.0)	97.3 (0.34)	0.00067	0.0026
DIBP	95.2 (2.5)	90.1 (0.80)	101 (4.9)	79.3 (0.35)	0.00092	0.0036
DBP	115 (1.5)	91.1 (0.90)	89.3 (4.1)	110 (0.69)	0.0023	0.0088
DMEP	113 (0.80)	102 (3.7)	113 (5.9)	81.8 (1.7)	0.0030	0.012
DIPP	90.2 (1.3)	96.3 (8.3)	110 (2.1)	90.4 (9.8)	0.0034	0.013
DPP	120 (0.90)	91.5 (0.70)	118 (7.1)	84.6 (1.2)	0.0019	0.0074
BBP	114 (3.6)	104 (0.20)	118 (5.7)	90.8 (0.63)	0.0045	0.018
DIHP	118 (4.0)	108 (2.3)	94.6 (0.80)	114 (8.4)	0.10	0.40
DEHP	109 (5.1)	96.8 (0.10)	99.6 (2.6)	83.7 (4.7)	0.0018	0.0072
DCHP	114 (2.4)	104 (13)	120 (3.7)	79.9 (4.9)	0.0011	0.0044
DPhP	104 (5.6)	106 (2.3)	111 (1.8)	91.1 (4.7)	0.0026	0.010
DNOP	120 (0.02)	102 (1.6)	139 (6.1)	86.4 (5.6)	0.0024	0.0096
DINP	110 (9.3)	89.3 (9.8)	87.7 (1.5)	108 (14)	0.10	0.40
DIDP	102 (15)	101 (0.20)	71.5 (15)	97.1 (15)	0.21	0.80
Musks	**Recoveries (baby wipe)**		**Recoveries (wet toilet paper)**		**LOD**	**LOD**
	10 µg/wipe	**100 µg/wipe**	**10 µg/wipe**	**100 µg/wipe**	**(µg g^{-1})**	**(µg/wipe)**
Cashmeran	105 (3.5)	94.3 (2.4)	96.8 (3.9)	82.6 (0.85)	0.0027	0.011
Celestolide	104 (3.6)	87.4 (0.80)	93.9 (3.5)	79.5 (0.05)	0.00082	0.0032
Phantolide	108 (1.5)	87.6 (0.40)	93.6 (6.4)	79.6 (0.74)	0.00082	0.0032
Musk Ambrette	98.8 (4.5)	82.4 (0.10)	92.7 (2.4)	109 (2.8)	0.0075	0.029

Table 6. Continuation

Musks	Recoveries (baby wipe)		Recoveries (wet toilet paper)		LOD	LOD
	10 µg/wipe	100 µg/wipe	10 µg/wipe	100 µg/wipe	(µg g^{-1})	(µg/wipe)
Traseolide	109 (0.60)	n.c	99.0 (1.8)	n.c	0.00082	0.0032
Galaxolide	120 (2.3)	86.9 (0.40)	107 (4.7)	82.4 (0.42)	0.0011	0.0044
Tonalide	113 (2.8)	86.3 (0.20)	101 (4.1)	81.6 (1.1)	0.0011	0.0042
Musk Moskene	106 (2.5)	82.6 (0.50)	112 (9.5)	110 (1.5)	0.0031	0.012
Musk Tibetene	115 (1.5)	83.3 (0.90)	113 (2.0)	109 (0.09)	0.0075	0.029
Ambrettolide	102 (12)	86.9 (0.70)	99.8 (5.2)	108 (0.21)	0.010	0.040
Musk Ketone	111 (0.30)	86.2 (2.1)	115 (5.3)	112 (1.3)	0.015	0.060

(---) Not calculated since initial concentration is higher than the spiked level (see **Table 7**)

3.3. Application to real samples

The validated method was applied to the analysis of twenty real commercial samples (13 baby wipes and 7 wet toilet paper), which represent a wide variety of these kind of baby care products. The analysis of the considered groups of cosmetic ingredients or additives is complex due to the own nature of the wipe samples and due to the fact that the analytes can be present from traces concentrations at the sub µg g^{-1} level up to several thousands of µg g^{-1}. The inclusion of injection blanks (after each sample analysis) and process blanks is mandatory.

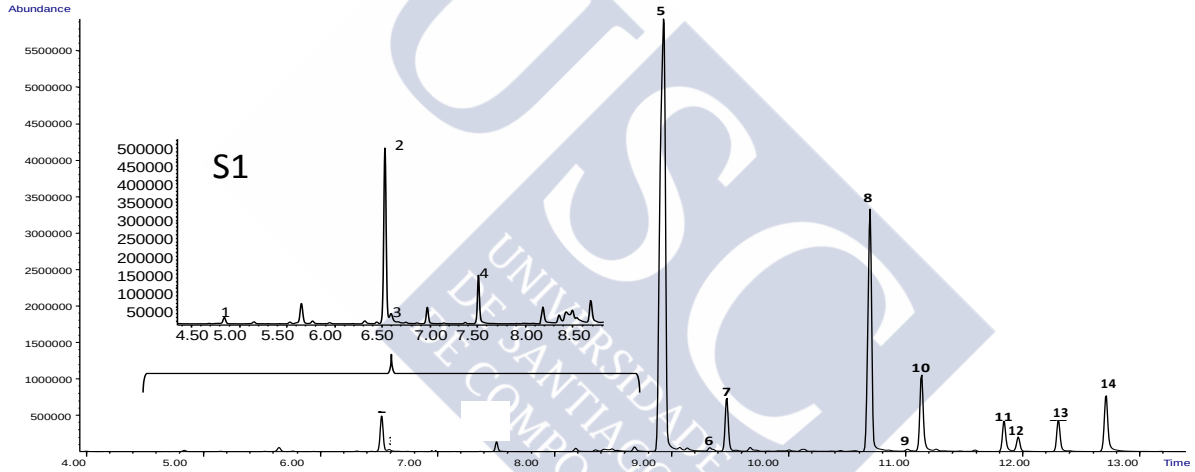
Table 7. Analysis of commercial baby wipes and wet toilet paper (µg g^{-1}).

	Baby wipes													Wet toilet paper						
Fragrance allergens	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**
Pinene	7.33			1.09									0.107							
Limonene	180	0.741	12.4	23.9	0.212	1.89			0.0669	0.0360	1.61		0.273		0.239		0.0760	0.262		2.67
Benzyl Alcohol	5.99		711	3.40		3.01	0.160	2446	0.0341	0.0448				1.67	2.81		0.0656		0.172	7.06
Linalool	76.0	32.8		59.9					1.10	1.04	0.810							0.492	0.698	14.6
Citronellol									1.82											
Citral	39.9			6.60																
Geraniol		12.7							0.311	0.250	1.21									11.1
Hydroxycitronellal	200										0.203									
Anise Alcohol																		11.8		56.4
Cinnamyl Alcohol								0.503			0.741						0.207			32.3
Coumarin		14.0																		77.0
α-isomethylionone		5.37				0.292		4.10			0.805									
Lilial®		0.0435	1.51	7.98			0.103													
Lyral®			0.932																	
Amylcinnamyl alcohol			0.470														1.29			
Farnesol			1.76																	
Hexylcinnamal		0.189	2.25			60.1														
Benzyl Benzoate		0.0613	0.0488					5.92			0.0585					0.0750				
Benzyl Salicylate			0.0617								0.168								0.0491	
Preservatives[a]	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**
PhEtOH	2459	1303	587	8368	4743	799	2412	2012	229	664	435	2297	727	2.68	2073	3427	19.5	3779	714	74.3
MeP	1213	11.3	0.230	3017	235	267		0.382							739		2.16			1387
BHT	0.602					0.638	0.0162					0.0302			0.0369	0.126	0.694	0.0311		
EtP	290	4.80		704	286	55.8									7.94				0.132	587
iPrP															8.66					
PrP	126	1.41		315	486	27.0									226					
IPBC														12.7						
iBuP	97.0	0.0934	1.48	247			2.71	2.03						0.113						
BuP	218	2.63		600		54.1														
Phthalates	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**
DEP	0.770	0.168	0.0285		0.0420	46.9	0.364				55.5	0.842	1.09	1.43	1.09	0.802	2.26	0.471	3.24	412
DIBP		0.0763	0.0404			0.0446	0.189					0.0481		0.102		0.155		0.111	0.0907	
DBP		0.130	0.136	0.176			0.130					0.0556		0.0359	0.132	0.0980		0.111		
DEHP			0.0357		0.161				0.0955				0.0351		0.225	0.126			0.0277	
DCHP									0.108											
Musks	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**
Cashmeran								0.183							0.480				1.85	
Galaxolide		0.735				10.8						0.332	0.0508		0.0610			0.151	0.0414	21.4
Tonalide											0.865					0.0270			0.0641	

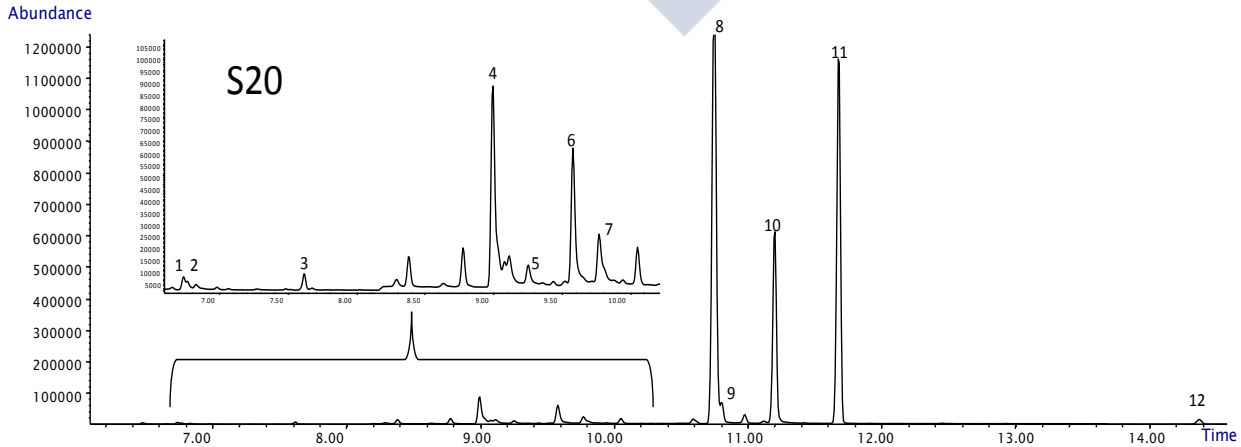
Thirty-six of the 65 targets were found in the analyzed samples. Results are shown in **Table 7** (expressed as μg g^{-1}) and table S1 (expressed as μg per wipe). The recoveries of benzyl alcohol-d_7, MeP-d_4 and DEHP-d_4 (surrogate standards) were satisfactory, with values between 84-111%, 85-112%, and 74-119%, respectively. **Figure 5** shows the chromatograms of two real samples, a baby wipe (S1), and a wet toilet paper (S20), highlighting the presence of PhEtOH, and MeP with concentration levels up to 0.25% (2500 μg g^{-1}).

Fragrance allergens

Nineteen of the twenty-six fragrance allergens were detected in the samples at concentrations between 0.0341-2446 μg g^{-1}, and all samples except S12 (labeled as fragrance free) contained fragrance allergens. Limonene, benzyl alcohol and linalool were the most frequently found (45-70% of the samples) with values up to 2446 μg g^{-1} (0.2%). Also geraniol, and benzyl benzoate were detected in 5 samples at concentration levels below 13 μg g^{-1}. Regarding the number of compounds by cosmetic sample, S3 contained 9 of the target fragrance allergens, highlighting benzyl alcohol concentration (711 μg g^{-1}), whereas the other samples contained between 1-8 target analytes. All samples fulfilled European restrictions regarding this group of ingredients.



1: pinene, 2: limonene, 3: benzyl alcohol, 4: linalool, 5: phEtOH, 6: citral, 7: hydroxycitronellal, 8: MeP, 9: BHT, 10: EtP, 11: DEP, 12: PrP, 13: iBuP, 14: BuP



1: limonene, 2: benzyl alcohol, 3: linalool, 4: phEtOH, 5: geraniol, 6: anise alcohol, 7: cinnamyl alcohol, 8: MeP, 9: coumarin, 10: EtP, 11: DEP, 12: galaxolide

Fig. 5. Chromatograms for samples S1 and S20 (see concentrations in **Table 7**).

Preservatives

In the case of preservatives, phenoxyethanol was found in all samples, at very high concentration in many of them (>1300 μg g^{-1}, 0.13%), although these concentration levels do not exceed the maximum concentration permitted by the European legislation (1%). In the case of parabens, 6 of the 7 targets were detected. The most common were MeP and EtP (50 and 40% of the samples, respectively) at concentrations up to 0.3%. PrP and BuP (will be banned form April 2015) were also found in 6 and 4 samples, respectively. iPrP and iBuP, which have been recently banned for their use in cosmetics and personal care products (the prohibition entered into force in October 2014), have been detected in 7 and 1 samples, respectively, at concentration between 0.0934-247 μg g^{-1}. IPBC, banned in products intended for children under 3 years was detected in 1 sample (S14) at 12.7 μg g^{-1} (in this case, the label indicates do not use this product for children under 3 years). BHT was detected in eight samples at low levels (<0.694 μg g^{-1}). The maximum number of target preservatives per sample was seven (S1), highlighting MeP and phenoxyethanol at concentrations higher than 0.1%. Trace levels of preservatives at the low and sub μg g^{-1} have been detected. Their presence may be caused by their use during manufacture of other cosmetics or as an impurity of other cosmetic ingredients, since those very low concentrations do not have antimicrobial effect.
All samples comply with European legislation in terms of maximum concentration permitted since phenoxyethanol and parabens (in this last case the samples were acquired before October 2014) do not exceed these levels (see **Table 1**) and the presence of IPBC is indicated in the label. Although European Regulation does not require the inclusion of preservatives in the personal care products list of ingredients, in general, all of them were declared.

Musks

Only 3 of the 10 studied musks were detected in the samples. Galaxolide was the most found, 40% of the samples, at levels below 0.7 μg g^{-1}, except S6 and S20 with concentrations of 10.8 and 21.4 μg g^{-1}, respectively. Tonalide and cashmeran were detected in 3 samples, at low levels (< 1.85 μg g^{-1}). Most samples only contain galaxolide, excluding S19 that presents cashmeran, galaxolide and tonalide at concentrations between 0.044-1.85 μg g^{-1}. Only tonalide presents restrictions about its maximum concentration permitted in personal care products and, in all cases, concentrations were below the established limit.

Phthalates

Five phthalates were detected in 18 samples. The most found was DEP (in 80% of the samples), highlighting its high concentration in S20 (412 μg g^{-1}). The banned DEHP, and DBP were also detected in 13 samples at low levels (<0.225 μg g^{-1}). DCHP only was found in one sample at 0.108 μg g^{-1}.

4. CONCLUSIONS

A pressurized liquid extraction followed by gas chromatography–mass spectrometry method has been developed for the determination of fragrance allergens, preservatives, phthalates, and musks in baby wipes and wet toilet paper intended for children. Twenty-five of the 65 target analytes are banned or subjected to restrictions according to European Legislation (EC No 1223/2009). The PLE/GC-MS method was optimized by means of experimental design in order to

select optimal PLE conditions (MeOH, 110°C and 5 minutes). The method exhibited a satisfactory performance in terms of linearity, sensitivity, accuracy and precision, with mean recoveries of 90 % and RSD values generally below 10%. IDLs were well below the established regulated limits. Finally, the validated method was applied to a high number of real samples available at the market.

Most of the target substances were found in the samples at concentration levels from the sub parts per million to the parts per million. Nineteen of the 26 target fragrance allergens were detected in the analyzed samples. Limonene, benzyl alcohol and linalool were the most frequently found (45-70% of the samples) with values up to 0.2%. In the case of preservatives, phenoxyethanol was detected in all samples at high concentrations in some cases (> 0.2% in 9 samples), MeP, and EtP were found in 40-50% of samples and the recently banned iPrP and iBuP, have been detected in seven and one samples, respectively, at concentration levels between 0.0934-247 μg g^{-1}. In the case of phthalates and musks, five and three target analytes were detected, respectively, including the forbidden phthalates DBP, and DEHP. In addition, 25% of the samples did not fulfill the labeling requirements for fragrance allergens. It is important to note that according to the last updates of the European legislation, 50% of the analyzed commercial samples would be prohibited from April 2015.

Acknowledgements

This research was supported by FEDER funds and projects CTQ2013-46545-P (Ministry of Economy and Competitively, Spain) and GPC2014/035 (Consolidated Research Groups Program, Xunta de Galicia).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at: http//dx.doi.org/10.1016/j.chroma.2015.01.049.

References

[1] J.D. Guin, J. Kincannon, F.L. Church, Am. J. Contact Dermatitis, 12 (2001) 189.

[2] A. Timmermans, S. De Hertog, K. Gladys, H. Vanacker, A. Goossens, Contact dermatitis, 57 (2007) 97.

[3] H.T. Shin, Pediatr. Clin. N. Am., 61 (2014) 367.

[4] G.C. Priestley, E. MeVittie, R.D. Aldridge, Pediatr. Dermatol., 13 (1996) 14.

[5] J. Nikolovski, G.N. Stamatas, N. Kollias, B.C. Wiegand, J. Invest. Dermatol., 128 (2008) 1728.

[6] T. Lavender, C. Furber, M. Campbell, S. Victor, I. Roberts, C. Bedwell, M.J. Cork, BMC Pediatr., 12 (2012) 59.

[7] G.N. Stamatas, N.K. Tierney, Pediatr. Dermatol., 31 (2014) 1.

[8] A. Makri, M. Goveia, J. Balbus, R. Parkin, J. Toxicol. Env. Health. B, 7 (2004) 417.

[9] P.J. Landrigan, L.R. Goldman, Health Affair., 30 (2011) 842.

[10] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast), in Off. J. Eur. Union L342/59., 2009.

[11] U.S. Food and Drug Administration. Available at: http://www.fda.gov/Cosmetics/ProductsIngredients/ingredients/ucm128042.htm# What_are_parabens (Accessed October 2014).

[12] Statutory order on restriction on import, sale and use of certain parabens in cosmetic products for children under 3 years. Danish Ministry of the Environment. Statutory Order on cosmetic products. 1217 of 11 October 2013. Available at:http://eng.mst.dk/media/mst/Attachments/Engelskpara benbekendtgrelse.pdf (Accessed October 2014).

[13] Agence nationale de sécurité du medicament et des produits de santé. Available at: http://ansm.sante.fr/S-informer/Points-d-information-Points-d-information/L-ANSM-recommande-de-restreindre-la-concentration-de-phenoxyethanol-dans-les-produits-cosmetiques-destines-aux-enfants-de-moins-de-3-ans-Point-d-information (Accessed September 2014).

[14] A. Salvador, A. Chisvert, Analysis of cosmetic products, Elsevier, Amsterdam, 2007.

[15] N. Cabaleiro, I. de la Calle, C. Bendicho, I. Lavilla, Anal. Methods, 5 (2013) 323.

[16] L.H. Wang, Anal. Methods, 5 (2013) 316.

[17] N. Cabaleiro, I. de la Calle, C. Bendicho, I. Lavilla, TrAC Trends Anal. Chem., 57 (2014) 34.

[18] M. Llompart, M. Celeiro, J. Pablo Lamas, L. Sanchez-Prado, M. Lores, C. Garcia-Jares, J. Chromatogr. A, 1293 (2013) 10.

[19] L. Sanchez-Prado, G. Alvarez-Rivera, J.P. Lamas, M. Llompart, M. Lores, C. Garcia-Jares, Anal. Methods, 5 (2013) 416.

[20] U. Blume-Peytavi, M. Hauser, L. Lünnemann, G.N. Stamatas, J. Kottner, N. Garcia Bartels, Pediatr. Dermatol., 4 (2014) 1.

[21] M.W. Chang, R. Nakrani, Pediatrics, 133 (2014) 434.

[22] A. Goossens, Exp. Rev. Dermatol., 6 (2011) 591.

[23] C. Liao, K. Kannan, Sci. Total Environ., 475 (2014) 8.

[24] P.D. Tzanavaras, T.D. Karakosta, P.G. Rigas, D.G. Themelis, A. Zotou, Cent. Eur. J. Chem., 10 (2012) 1459.

[25] L. Sanchez-Prado, J.P. Lamas, M. Lores, C. Garcia-Jares, M. Llompart, Anal. Chem., 82 (2010) 9384.

[26] J.P. Lamas, L. Sanchez-Prado, C. Garcia-Jares, M. Lores, M. Llompart, J. Chromatogr. A, 1217 (2010) 8087.

Appendix A. Supplementary data

Table S1. Analysis of commercial baby wipes and wet toilet paper (µg/wipe).

	Baby wipes												Wet toilet paper							
Fragrances allergens	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**
Pinene	18.2			4.28									0.690							
Limonene	448	3.96	107	93.4	0.777	10.2			0.591	0.201	7.82		1.75		0.740		0.437	0.948		17.0
Benzyl Alcohol	14.9		6135	13.3		16.2	0.957	7164	0.301	0.250				5.85	8.67		0.377		0.502	45.0
Linalool	189	175		235					9.67	5.78	3.92							1.78	2.03	93.1
Citronellol									16.1											
Citral	99.3			25.8																
Geraniol		67.7							2.75	1.40	5.87									70.5
Hydroxycitronellal	498										0.986									
Anise Alcohol																		42.6		359
Cinnamyl Alcohol								1.47			3.61						1.19			206
Coumarin		74.6																		491
α-isomethylionone		28.7				1.57		12.0			3.92									
Lilial®		0.232	13.0	31.3			0.616													
Lyral®			8.04																	
Amylcinnamyl alcohol														1.65			7.40			
Farnesol			15.2																	
Hexylcinnamal		1.1	19.4			323														
Benzyl Benzoate		0.330	0.421					17.4			0.285					0.291				
Benzyl Salicylate			0.530								0.816								0.143	
Preservatives[a]	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**
PhEtOH	6116	6961	5065	32770	17392	4292	14464	5893	2026	3705	2117	16500	4664	9.38	6406	13297	111	13673	2079	473
MeP	3017	60.3	1.99	11815	861	1433		1.12							2283		12.4			8837
BHT	1.49					3.43	0.0970					0.234			0.114	0.489	3.99	0.112		
EtP	721	25.6		2757	1048	300									24.5				0.384	3738
iPrP															26.7					
PrP	312	7.55		1235	1781	145									697					
IPBC														44.4						
iBuP	240	0.499	12.8	967			22.8	5.96						0.395						
BuP	541	14.0		2350		291														
Phthalates	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**
DEP	1.91	0.895	0.246		0.155	252	2.18				270	6.05	7.02	4.99	3.38	3.11	13.0	1.70	9.43	2628
DIBP		0.407	0.349			0.240	1.13					0.350		0.358		0.601		0.400	0.264	
DBP		0.697	1.17	0.689			0.78					0.399		0.126	0.408	0.380		0.421		
DEHP			0.308		0.591				0.843				0.225		0.696	0.489			0.0807	
DCHP									0.952											
Musks	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**
Cashmeran								0.536							1.48				5.39	
Galaxolide		3.93				57.9						2.39	0.326		0.188			0.546	0.121	137
Tonalide											4.21					0.105			0.187	

[a] Parabens concentration expressed as acid.

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2. Determinación de fungicidas en vino y subproductos de vinificación

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El sector vitivinícola es uno de los pilares fundamentales de la agricultura en Galicia, donde existen más de 25.000 hectáreas de terreno dedicadas al cultivo de la vid bajo 5 Denominaciones de Origen (D.O. Ribeiro, D.O. Rías Baixas, D.O. Ribeira Sacra, D.O. Monterrei, D.O. Valdeorras).

La climatología gallega (abundantes lluvias y temperaturas extremas) favorece la proliferación de ciertos agentes fitopatógenos que pueden provocar pérdidas en la productividad de los cultivos, por lo que es necesario aplicar tratamientos fitosanitarios para evitar o minimizar los daños ocasionados. Como ya se ha comentado, los fungicidas son compuestos químicos muy estables por lo que pueden no ser eliminados completamente durante el proceso de vinificación; por ello, se hace necesario el desarrollo de métodos analíticos rápidos y fiables para la determinación de estos compuestos en los vinos y en los subproductos que se generan durante su elaboración.

El bagazo es el principal residuo que genera la industria vitivinícola. De los 63 millones de toneladas de uvas que se produjeron en el mundo en 2012, el bagazo representa el 30%. Este subproducto es una importante fuente de compuestos fenólicos. Por ello, en los últimos años se está despertando un interés creciente, tanto por razones económicas, como medioambientales para reutilizar estos residuos como fuente de compuestos bioactivos, los cuales pueden ser empleados en la industria farmacéutica, cosmética o alimenticia. Llama la atención que hasta la realización de esta Tesis no existía ningún método analítico para la determinación de fungicidas en este producto.

Por lo tanto el ***principal objetivo*** del ***Capítulo II*** de esta Tesis fue desarrollar ***métodos analíticos para el estudio simultáneo de los fungicidas empleados habitualmente en el tratamiento de los viñedos gallegos y que pudiese ser aplicado a muestras de bagazo de uva blanca y a vinos blancos.*** La técnica de extracción seleccionada dependió de la naturaleza sólida o líquida de la muestra.

Para el análisis de vinos, la microextracción-emulsificación asistida por ultrasonidos (*ultrasound-assisted emulsification-microextraction*, USAEME) fue la técnica de extracción seleccionada debido a su eficacia, rapidez y bajo consumo de disolvente orgánico (del orden de µL). Esta técnica puesta a punto por el grupo de investigación en el que se ha desarrollado este trabajo en 2008, ha sido aplicada con éxito a la determinación de un gran número de analitos principalmente en aguas, sin embargo, nunca hasta ahora había sido aplicada al análisis de fungicidas en vinos.

Para el caso del bagazo, el primer planteamiento que se tanteó fue el de emplear una técnica de extracción muy sencilla y de bajo coste como es la extracción asistida por ultrasonidos (*ultrasound assisted extraction, UAE*). Este enfoque varió al comparar esta técnica con PLE, ya que con esta última, la respuesta fue significativamente mayor para todos los fungicidas estudiados, llegando a cuadriplicarse la señal para algunos de ellos. Por ello, todo el desarrollo y validación del método se llevó a cabo empleando PLE como técnica de extracción. Además, se realizó una comparativa entre la aplicación de GC-MS y GC-MS/MS, obteniéndose en este último caso IDLs y LODs de hasta 2 órdenes de magnitud menores que empleando solamente MS, lo que supone un gran avance para detectar trazas de fungicidas en bagazo.

Las condiciones experimentales de ambos métodos analíticos fueron optimizadas mediante diseños experimentales para obtener la máxima eficacia de extracción; posteriormente ambos

métodos fueron validados mediante herramientas estadísticas en términos de linealidad, exactitud y precisión y por último se aplicaron a muestras de bagazo y vinos blancos de las 5 Denominaciones de Origen gallegas.

Por lo tanto, en resumen, la segunda parte de esta Tesis Doctoral se ha centrado en el desarrollo de métodos de análisis rápidos y robustos para determinar fungicidas tanto en vino como en subproductos de vinificación, lo que ha llevado a los siguientes estudios que se discutirán a continuación:

- Análisis rápido de fungicidas en vinos blancos del Noroeste de España mediante microextracción-emulsificación asistida por ultrasonidos y cromatografía de gases-espectrometría de masas (*Anal. Methods*, 6 (2014) 3108-3116).

- Determinación de fungicidas en bagazo de uva blanca mediante extracción con líquidos presurizados y cromatografía de gases-espectrometría de masas en tándem (*J. Chromatogr. A*, 1343 (2014) 18-25).

2.1. RAPID ANALYSIS OF FUNGICIDES IN WHITE WINES FROM NORTHWEST SPAIN BY ULTRASOUN-ASSISTED EMULSIFICATION-MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Analytical Methods, (Ed. Royal Society of Chemistry, United Kingdom)
ISSN: 1759-9660

Anal. Methods, 6 (2014) 3108-3116. **doi:10.1039/C3AY42285B**

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RAPID ANALYSIS OF FUNGICIDES IN WHITE WINES FROM NORTHWEST SPAIN BY ULTRASOUND-ASSISTED EMULSIFICATION-MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

A rapid, efficient and low-cost method based on ultrasound-assisted emulsification-microextraction (USAEME) and gas chromatography-mass spectrometry was developed for the analysis of several fungicides belonging to different chemical families, in white wines. The experimental procedure was optimized using factorial design to study the influence of the solvent type, extraction time, extraction temperature, sample, pH, and salt addition on the extraction efficiency. Under the selected conditions, compounds were extracted in 200 µL chloroform from 10 mL of wine sample in only 5 min at ambient temperature. The method was validated using real wine samples, with fungicide recoveries generally ranging from 70 to 115%, good intra- and inter-day precision (relative standard deviation (RSD) values ≤12%), and limits of detection at the sub-ng per millilitre level (LOD≤0.1 ng mL^{-1}). The application of the method to varietal wines belonging to different brands showed the presence of fungicides in all samples at concentrations ranging from 1 to 700 ng mL^{-1}, with a minimum of two and a maximum of seven chemicals per sample.

1. INTRODUCTION

Fungicides are extensively used in viticulture. Fungicide application is especially important in rainy regions, where the proliferation of powdery mildew (*Erysiphe necator*, formerly *Uncinula necator*), downy mildew (*Plasmopara viticola*), and grey rot (*Botrytis cinerea*) is associated with high levels of precipitations. However, besides their beneficial effects on grape crop preservation, fungicides may be toxic for both humans and other living organisms. Fungicides levels are regulated in grapes [1], but not in wines, although systemic fungicides can remain after the wine-making process and be found in the final wine. A recent survey on bottled wines demonstrated the presence of pesticides, mainly fungicides in 90% of the wine samples, some of them containing up to 9 different chemicals [2]. Due to their toxicity and the increasing consumer concern about the use of all classes of pesticides in foods and beverages, the presence of fungicides in wines must be controlled.

In recent years, great efforts have been made to develop analytical methodologies to achieve high sensitivity as well as procedural simplicity in the analysis of fungicides, among other pesticides, in all kinds of environmental and agri-food samples. In wines, most methods are based on gas chromatography coupled to mass spectrometry (GC-MS) analysis of the extracts obtained by solid phase extraction (SPE) [3-7], as well as by microextraction techniques such as dispersive liquid-liquid microextraction (DLLME) [8], solid phase microextraction [9,10], bar adsorptive microextraction [11], membrane-assisted solvent extraction [12], and sorptive microextraction using disposable silicone sorbents [13]. Ultrasound-assisted emulsification microextraction (USAEME) [14] has recently been used in one of the most successful microextraction techniques, finding application in the analysis of a broad range of analytes in water samples. This technique is based on the emulsification of a microvolume of organic solvent in an aqueous sample by ultrasound radiation, and further separation of the two liquid phases. A summary of the applications, which include both inorganic and organic compounds analysis, the use of high and low density solvents, as well as different devices configurations, can be found in recent reviews [15,16]. The advantages and the increasing extension of USAEME to matrices other than water have also been reported, although USAEME has scarcely been applied. The determination of haloanisoles and volatile phenols [17], and organic sulfur compounds [18] has been described, and very recently, a surfactant-enhanced emulsification-microextraction led to a floating organic droplet analyzed by high performance liquid chromatography with diode array detection for the determination of six fungicides in juices and red wine samples [19].

The aim of the present work is to develop an efficient and rapid USAEME and gas chromatography-mass spectrometry method to determine several broadly used fungicides in white wines. We have focused our attention on eight fungicides widely used in Galicia (Northwestern Spain), characterized by a precipitation regime distinctive to the European Atlantic regions. These compounds were: benalaxyl, cyprodinil, dimethomorph, iprodione, kresoxim-methyl, (R)-metalaxyl, myclobutanil, and procymidone. The selected configuration was previously proposed [14-20] and involves the use of conical centrifuge tubes, since these are very simple and low-cost devices, allowing the easy handling of both the sample and the micro extracts. The influence of extraction solvent, sample pH, extraction time and temperature, as well as the salting-out effect, were studied

and optimized using experimental design tools. The developed method was validated and applied to wines elaborated with typical white grape varieties in different protected production areas of the Northwest Spanish region.

Table 1. Some physic-chemical characteristics, maximum residue limit (MRL), retention times, and selected ions for the compounds.

Compound	IUPAC name	CAS number	pK_{ow}	pKa	Boiling point (°C)	MRL USA [4] (grapes, mg Kg^{-1})	MRL EU [1] (grapes, mg Kg^{-1})	Retention time (min)	Quantifier ion(m/z)	Qualifier ions (m/z)
Benalaxyl	methyl N-phenylacetyl-N-2,6-xylyl- DL-alaninate	71626-11-4	3.40	1.52	468	-	0.3	13.11	148	91, 206
Cyprodinil	4-cyclopropyl-6-methyl-N-phenylpyrimidin-2-amine	121552-61-2	3.00	4.22	406	3	5	10.94	224	225, 210
Dimethomorph	(E,Z)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl]morpholine	110488-70-5	2.68	-1.19	585	3	3	18.88,19.46	301	165, 387
Iprodione	3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide	36734-19-7	3.00	-2.23, 9.19	545	60	10	13.92	314	187, 245
Kresoxim-methyl	methyl (E)-methoxyimino(o-tolyloxymethyl)phenylacetate)	143390-89-0	3.40		429	1	1	12.08	131	116, 206
(R)-Metalaxyl	Methyl (R)-2-(2,6-dimethylphenyl) methoxyacetyl amino propionate	70630-17-0	1.71	1.41	394	2	1	9.89	206	160, 192
Myclobutanil	2-p-chlorophenyl-2-(1H-1,2,4-triazol-1-ylmethyl)hexanenitrile	88671-89-0	2.94	2.30	465	1	1	12.07	179	206,150
Procymidone	N-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide	32809-16-8	3.08	-2.67	478	5	0.01	11.25	283	96, 285

2. EXPERIMENTAL

Reagents and materials

Benalaxyl (98.0%), cyprodinil (97.5%), dimethomorph (98%), iprodione (97.5%), kresoxim-methyl (98.0%), (R)-metalaxyl (99.5%), myclobutanil (97.5%), procymidone (99.5%), and 2,4,6-trichlorobiphenyl (PCB 30, 99.0%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany); chlorobenzene (99.9%), tetrachloroethylene (99.9%), and 1,1,1-trichloroethane (>99.5%) were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany); chloroform and carbon tetrachloride were obtained from Merck (Darmstadt, Germany); and dichloromethane from VWR (Mollet del Vallés, Barcelona, Spain). Sodium chloride was provided by Sigma-Aldrich Chemie GmbH. All solvents and reagents were analytical grade.

Wine samples (vintage 2010) were elaborated under different quality protected geographical brands (Rias Baixas, Ribeiro, Valdeorras, Betanzos) from monovarietal white grapes (Albariño, Branca lexitima, Caiño, Godello, Loureiro, Torrontés, and Treixadura). For the analysis, samples were filtered through 0.22 µm Millipore GV membrane filters (Billerica, MA, USA).

Ultrasound-assisted emulsification–microextraction (USAEME)

Aliquots of 10 mL wine samples were placed in 15 mL conical-bottom glass centrifuge tubes, and 1 g of sodium chloride, and 200 µL of chloroform containing 50 ng mL^{-1} of PCB 30 (internal standard) were added. The tube was then immersed into a 5 L-ultrasonic water bath (Selecta, Barcelona, Spain) in such a way that the level of both liquids (bath and sample) was the same. Extractions were performed at 40 kHz of ultrasound frequency and 110 W of power for 5 min, resulting in an emulsion of chloroform in water that was then disrupted by centrifugation at a relative centrifugal force of 2220g (10 min). The organic phase at the bottom of the conical tube was removed by using a 100 µL Hamilton syringe (Reno, NV, USA) and transferred to a 250 µL glass insert placed in a 1.8 mL gas chromatography vial. The obtained extracts were stored at -20°C prior to GC-MS analysis. Working solutions of the analytes in chloroform were prepared for quantification purposes.

Gas chromatography–mass spectrometry

The GC-MS analysis was performed using an Agilent 7890A (GC) coupled to an Agilent 5975C inert triple axis mass spectra detector (MSD), with an Agilent 7693 autosampler from Agilent Technologies (Palo Alto, CA, USA). The temperatures of the transfer line, the quadrupole, and the ion source were set at 290, 150, and 230°C, respectively. The system was operated by Agilent MSD ChemStation E.02.00.493 software. Separation was carried out on a SLB-5MS capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness). Helium (purity, 99.999%) was employed as a carrier gas at a constant column flow of 1.0 mL min^{-1}. The GC oven temperature was programmed from 100°C (held 2 min) to 200°C at 15°C min^{-1}, to 260°C at 10°C min^{-1}, and finally to 290°C (held 10 min) at 20°C min^{-1}. Pulsed splitless mode was used for injection (30 psi, held 1.2 min). After 1 min, the split was opened at a flow of 75 mL min^{-1} and the injector temperature was kept at 260°C. The injection volume was 1 µL.

The MSD operated in selected ion monitoring mode (SIM), monitoring at three ions per compound (see **Table 1**). The electron multiplier was set at a nominal value of 1470 V.

3. RESULTS AND DISCUSSION

Optimization of the GC-MS analysis

The GC analysis was optimized to achieve an efficient separation of the target fungicides in less than 20 min. The MS detection was performed in SIM mode, by selecting three ions per compound (see **Table 1**) in order to assure the identification of the chromatographic peaks. The most abundant ions in the corresponding spectra were used for quantification of the compounds (**Table 1**). **Figure 1** shows the chromatogram of a standard mixture of the compounds (1 µg mL^{-1}) including PCB-30 added as IS (0.05 µg mL^{-1}).

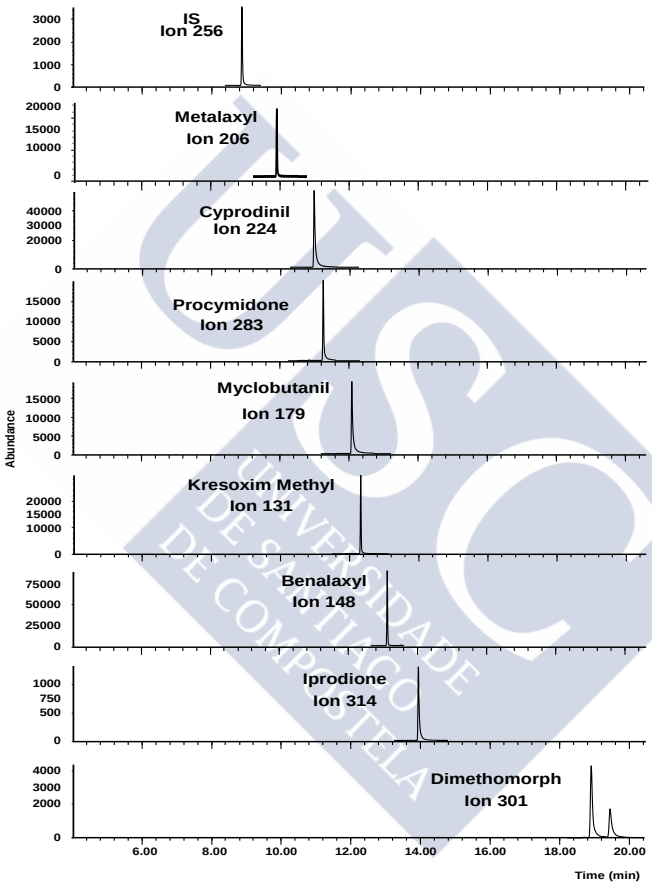

Fig. 1. GC-MS (SIM) chromatogram of a standard mixture of fungicides (1000 ng mL^{-1})

Optimization of the USAEME

Parameters that could potentially influence the efficiency of the analytical procedure were studied using a screening design. The extraction solvent, the sample pH, the extraction time and the temperature, as well as the salting-out effect, were considered for optimization. The selection of an appropriate solvent is an important parameter for USAEME processes. Based on previous studies [14,22], several solvents were initially considered: chloroform, carbon tetrachloride, chlorobenzene, trichloroethane, and tetrachloroethylene. The first experiments demonstrated that the best responses for all compounds were obtained using

chloroform. Carbon tetrachloride and trichloroethane showed similar responses, about 30-50% lower than those obtained with chloroform. Tetrachloroethylene was discarded since some compounds were not extracted, whereas chlorobenzene was discarded due to the presence of distorted chromatographic peaks. Thus, both chloroform and carbon tetrachloride were selected for the experimental design in an attempt to achieve the highest extraction efficiency for the compounds. The effect of sodium chloride addition was evaluated at two concentration levels in the sample, 0% (no addition), and 10% (w/v). Since solubility of sodium chloride is lower in wine than in water due to the ethanol content of wine, salt concentrations higher than 10% were not considered in order to avoid precipitation of the salt. The pH of wine usually ranges from 3 to 3.5. Two pH levels were studied for extraction optimization: the pH of unmodified wine (measured values for the samples considered ranged from 3.21 to 3.28) and pH 5. The effect of extraction temperature was evaluated at 25°C and 50°C. Two extraction times were examined: 5 and 15 min.

The selected optimization strategy consisted on a fractional screening design consisting of a 2^{5-1}design and two central points, involving a total of 18 experiments. Factor levels and the corresponding identification keys are summarized in **Table 2**. The selected design allowed us to determine which factors had a statistically significant effect, as well the significant interactions between factors. Experiments were performed using 10 mL aliquots of wine spiked with the analytes at a concentration of 20 ng mL^{-1}. Numerical analysis of data resulting from the experimental design was carried out using the Statgraphics XV Centurion statistical software package (Manugistics, Rockville, MD, USA).

Table 2. Factors and levels selected for the experimental design optimization

		Factor level	
Factor	Key	Low (-)	High (+)
NaCl (%)	A	0	10
pH	B	3[a]	5
Temperature (ºC)	C	25	50
Time (min)	D	5	15
Solvent type	E	Chloroform	Carbon Tetrachloride

[a]pH=3 in this table represents the unmodified pH of the wine (measured value in samples =3.21-3.28)

The analysis of variance (ANOVA) results are shown in **Table 3**. The ANOVA information can be clearly shown in Pareto charts (**Figure 2**), in which the length of each bar is proportional to the absolute value of its associated standardized effect, and the vertical line represents the statistically significant bound at the 95% confidence level. **Figure 3**, shows the main effects plots, with lines drawn between the low and the high levels of the corresponding factors. The length is proportional to the magnitude of the effect of the extraction process, and the sign of the slope indicates the level that produces the highest response.

The type of solvent and the addition of NaCl were the most important factors and their influence on extraction is clearly appreciated. For two of the studied compounds (kresoxim

methyl and procymidone) the pH of the sample was also significant. The extraction time and the extraction temperature were not significant for any of the compounds. Higher extraction efficiency was observed for all compounds on addition of sodium chloride.

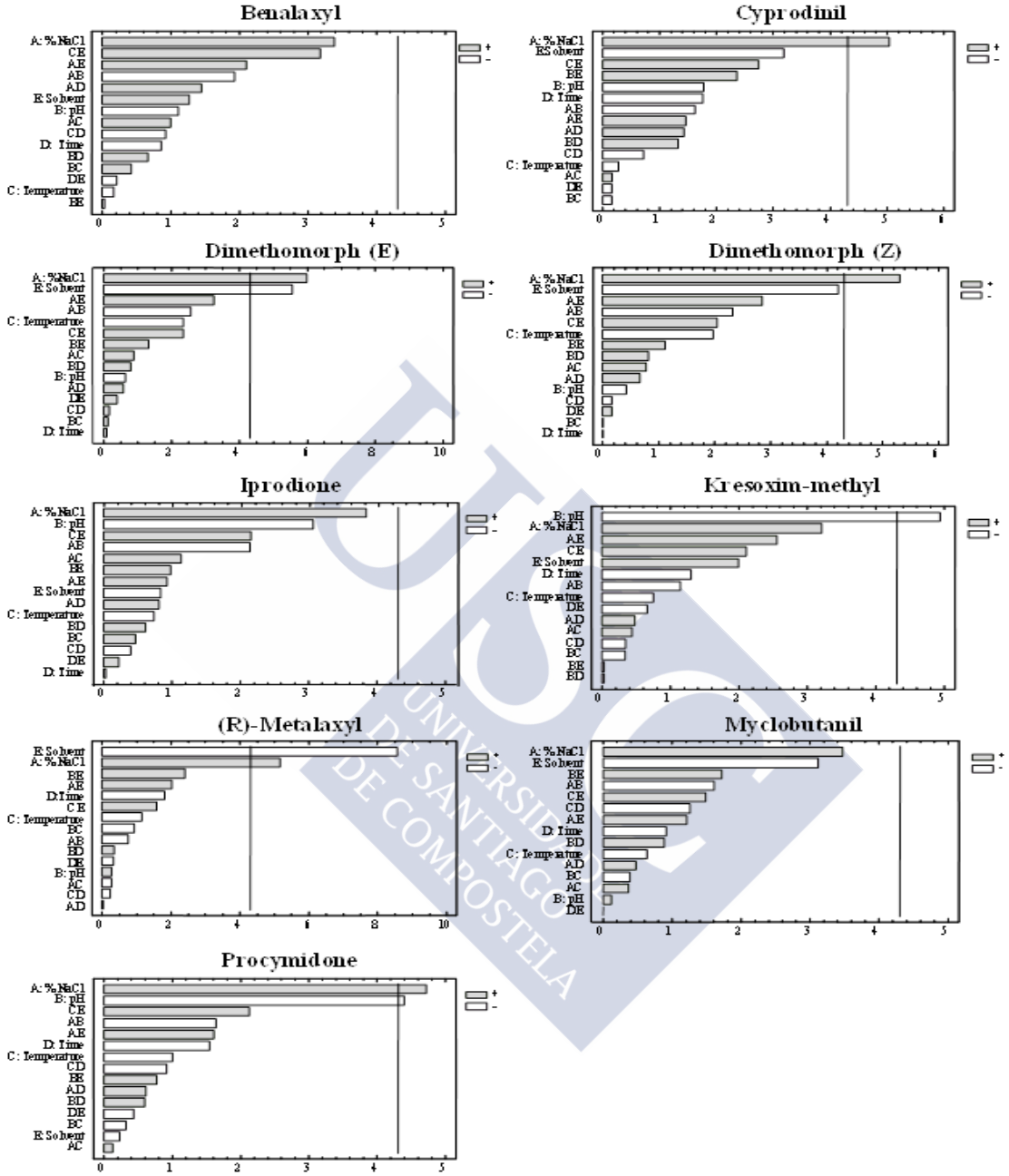

Figure 2. Pareto charts showing the significant factors (95%) for the compounds (see factor codes in **Table 2**).

The use of chloroform increased the extraction for all compounds, with the exception of kresoxim-methyl and benalaxyl, for which carbon tetrachloride showed better results, although the type of solvent was not significant for both these compounds ($p > 0.05$) (**Table 3**). All fungicides were extracted better at the natural pH of wine, and this was a significant factor in the extraction of procymidone and kresoxim-methyl. Although the extraction time and temperature were not significant ($p>0.05$) for any of the compounds, better results were

generally obtained when ultrasound was applied for 5 min at room temperature. The interactions between the main factors were not significant for any of the compounds.

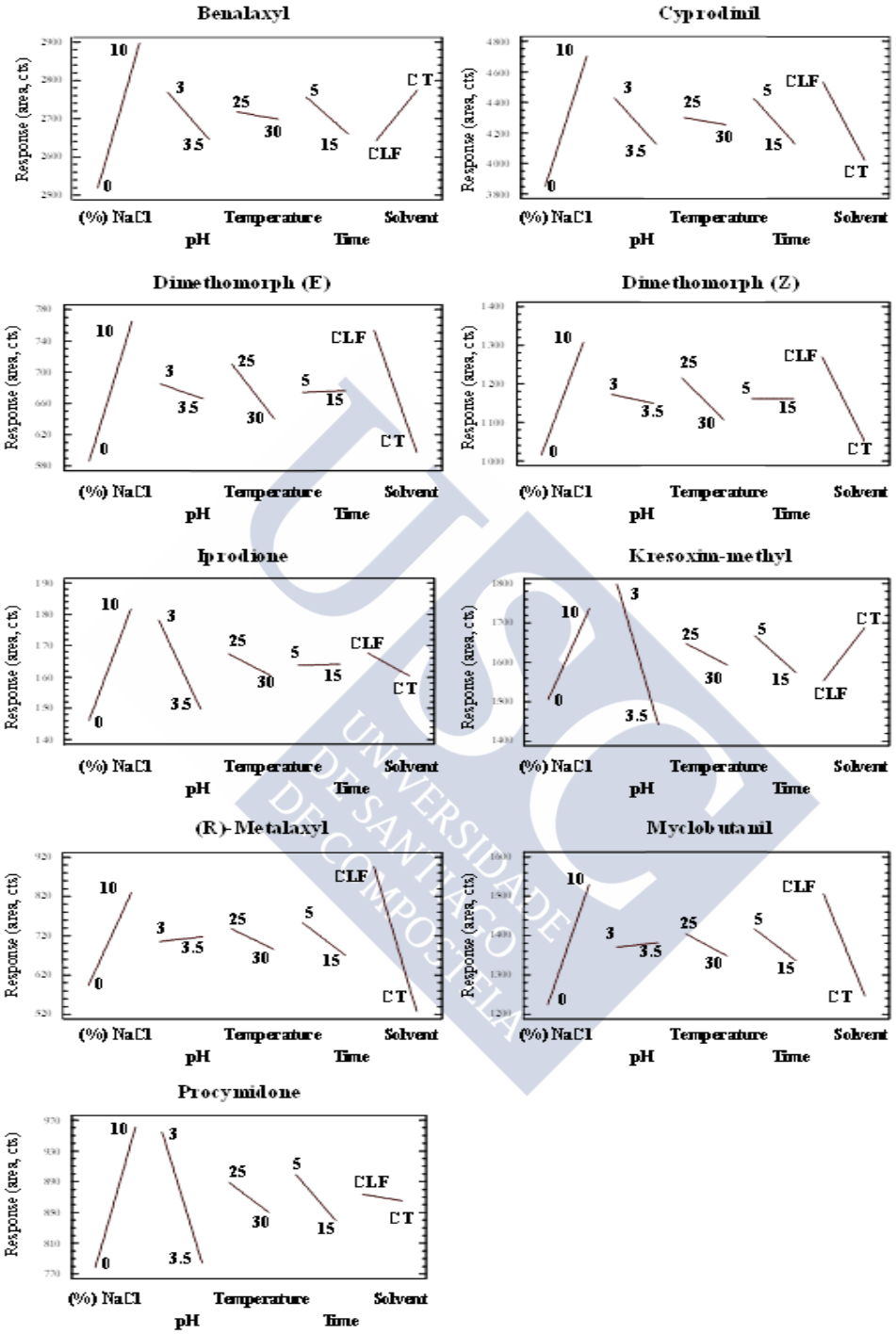

Figure 3. Main effects plots (CLF: chloroform; CT: carbon tetrachloride).

In view of the results of the experimental design, the general conditions for the simultaneous USAEME of the compounds from wine samples were selected as follows: addition of

10% sodium chloride to the sample, and extraction at room temperature with 200 µL chloroform employing a sonication time of 5 min.

Table 3. F ratios and p values obtained from the ANOVA of the 2^{5-1} screening design

Compound	NaCl (%) (A)		pH (B)		Temperature (ºC) (C)		Time (min) (D)		Solvent type (E)	
	F	p[a]	F	p	F	P	F	p	F	p
Benalaxyl	11.50	0.077	1.24	0.382	0.03	0.882	0.75	0.478	1.62	0.331
Cyprodinil	25.39	***0.037***	3.15	0.218	0.08	0.808	3.12	0.219	10.19	0.086
Dimethomorph (E)	35.89	***0.027***	0.41	0.587	5.52	0.143	0.01	0.936	30.87	***0.031***
Dimethomorph (Z)	28.19	***0.034***	0.17	0.717	3.91	0.187	0.00	0.997	17.70	0.052
Iprodione	14.77	0.061	9.44	0.092	0.55	0.537	0.00	0.970	0.70	0.490
Kresoxim-methyl	10.29	0.085	24.40	***0.039***	0.57	0.529	1.70	0.322	3.98	0.184
(R)-Metalaxyl	26.75	***0.035***	0.07	0.810	1.34	0.367	3.30	0.211	73.59	***0.013***
Myclobutanil	12.05	0.074	0.02	0.914	0.41	0.587	0.84	0.457	9.74	0.089
Procymidone	22.30	***0.042***	19.37	***0.048***	1.02	0.419	2.40	0.261	0.05	0.837

[a] Significant p values are shown in bold italics

Method performance

The linearity of the GC-MS was tested using standards prepared in chloroform at concentrations ranging between 10 ng mL^{-1} (20 and 50 ng mL^{-1}, depending on the compound) and 2000 ng mL^{-1} with 8 concentration levels and three replicates per level. Correlation coefficients from 0.9968 to 0.9999 were obtained for the compounds (**Table 4**). Instrumental detection limits (IDL) were estimated for a signal-to-noise ratio of 3 (S/N=3), and the values ranged from 0.50 to 2.90 ng mL^{-1}. The intra-day (n=3) and inter-day (n=6) of several spiked concentrations were evaluated (**Table 4**). The intra-day RSD results ranged from 0.1 to 11.5%, and the inter-day RSD varied from 2.2 to 11.8% (higher values, 14.0-21.7% were obtained for dimethomorph).

The performance of the proposed USAEME-GC-MS method was evaluated in terms of accuracy, precision, and limits of detection and quantification. Accuracy was evaluated using samples of white wines elaborated with three different grape varieties (Treixadura, Godello and Albariño) in which the concentration of the target fungicides were relatively low; samples were spiked at two concentration levels (2 and 20 ng mL^{-1}). Recoveries were calculated by dividing the difference between the measured concentrations for the spiked and non-spiked samples. As can be seen in **Table 5**, recoveries were satisfactory for all compounds, with values generally ranging from 70 to 115%. No differences related to the grape variety were observed. Recoveries of kresoxim-methyl and benalaxyl were in the range of 54-67% for the higher concentration (20 ng mL^{-1}). In general obtained recoveries were comparable to those previously reported for different types of wines, included red ones. Among them, Perez-Ortega et *al*. [4] recovered between 70 and 120% of several of the target fungicides using SPE-LC-MS-TOF analysis, while Rodriguez-Cabo et *al*. [8] and Fontana et *al*. [6] recovered 80-120% using DLLME-GC-MS and SPE-LC-MS-TOF, respectively. The addition of

analyte protectants to the final extracts allowed Gonzalez-Rodriguez et *al*. [23] to avoid the matrix-induced response enhancement effect on quantitation of benalaxyl among some other fungicides using GC-ion trap-MS. The precision of the method was evaluated by calculating the relative standard deviations (RSD) at the same concentration levels (n=3). The results are shown in **Table 5**, and they RSDs were lower than 12%. Limits of detection (LOD) and limits of quantification (LOQ) were estimated using a wine sample fortified with the compounds at 2 ng mL^{-1}. LOD values (*S/N=3*) ranged from 0.019 ng mL^{-1} to 0.13 ng mL^{-1}, and LOQ values (*S/N= 10*) ranged from 0.062 to 0.39 ng mL^{-1} (**Table 5**). These limits are similar to those obtained by Fontana et *al*. [6] using SPE and LC-MS/MS, about one order of magnitude lower than those reported by Lagunas-Allué et *al*. employing SPE and GC-MS [5], and about two orders of magnitude lower than those obtained for red wine by Wang et *al*. using dispersive microextraction with ionic liquids and HPLC-DAD [24].

Table 4. Linearity, instrumental detection limits, and precision.

Compound	Linearity range (ng mL^{-1})	R	IDL (ng mL^{-1})	Repeatability (% RSD)						
				Intra-day(n=3)			Inter-day(n=6)			
				50	200	1000	50	100	200	1000
Benalaxyl	10 - 2000	0.9979	0.50	2.1	1.2	0.7	2.3	4.2	5.4	2.8
Cyprodinil	10 - 2000	0.9990	0.75	2.9	0.8	1.1	3.6	3.5	2.5	2.2
Dimethomorph (E)	50 - 2000	0.9996	2.80	7.4	9.9	0.5	21.7	20.2	10.5	5.6
Dimethomorph (Z)	50 - 2000	0.9994	1.95	10.2	8.0	2.4	16.9	14.0	8.8	6.8
Iprodione	50 - 2000	0.9994	2.90	11.5	3.6	2.0	9.2	11.8	5.4	2.5
Kresoxim-methyl	20- 2000	0.9978	1.40	1.3	1.0	0.4	2.6	4.4	4.1	2.7
(R)-Metalaxyl	20 - 2000	0.9992	1.30	2.3	0.9	0.9	6.0	5.1	4.8	2.4
Myclobutanil	50 - 2000	0.9968	2.50	8.6	4.7	1.3	11.5	6.7	3.7	5.4
Procymidone	10 - 2000	0.9999	0.95	3.6	0.8	0.1	3.3	2.7	2.5	2.3

Table 5. Recovery[a], limits of detection, and limits of quantification of the whole method.

Compound	Recovery (% RSD, n=3)								LOD (ng mL^{-1})	LOQ (ng mL^{-1})
	2 ng mL^{-1}				20 ng mL^{-1}					
	G2	A11	Tr3	Tr2	G2	A11	Tr3	Tr2		
Benalaxyl	79.3 (5.2)	66.9 (4.0)	101 (7.0)	76.2 (4.4)	66.9 (7.5)	58.0(3.5)	64.3 (4.4)	54.5 (0.3)	0.035	0.12
Cyprodinil	108 (8.8)	89.7 (3.0)	-	98.4 (14)	99.2 (11)	81.3 (4.1)	89.9 (5.7)	78.6 (2.7)	0.019	0.062
Dimethomorph (E)	100 (19)	-	-	-	89.7 (8.7)	117(4.0)	105 (8.2)	117 (8.9)	0.058	0.19
Dimethomorph (Z)	86.6 (17)	-	-	-	86.3 (9.6)	121(5.1)	113 (9.4)	102 (6.0)	0.047	0.16
Iprodione	112 (9.2)	102 (4.2)	-	110 (5.2)	99.3 (1.6)	94.8 (6.8)	100 (6.1)	93.5 (0.8)	0.10	0.33
Kresoxim-methyl	83.0 (6.2)	73.4 (4.3)	109 (4.8)	78.0 (5.9)	63.1 (5.4)	57.8 (3.0)	64.3 (3.6)	53.5 (0.5)	0.11	0.36
(R)-Metalaxyl	106 (7.4)	91.5 (1.9)	-	118 (3.7)	99.7 (3.6)	78.7 (5.6)	93.9 (9.3)	78.7 (1.9)	0.13	0.39
Myclobutanil	114 (8.7)	105 (4.1)	112 (11)	95.1 (0.3)	102 (10)	88.4 (4.5)	98.4 (5.1)	92.3 (1.6)	0.095	0.32
Procymidone	88.4 (6.1)	71.1 (2.9)	102 (4.6)	98.9 (3.5)	79.5 (11)	68.5 (4.6)	75.7 (3.9)	70.5 (1.4)	0.030	0.10

[a]Grape variety key, A: Albariño; Tr: Treixadura; G: Godello

Application to real samples

Finally, the proposed method was applied to the analysis of several white monovarietal wines (vintage 2010), all produced in the Spanish Northwestern region under quality protected geographical brands. The presence of fungicides was confirmed in all the samples (**Table 6**) at concentrations below the regulated MRL values for grapes in the USA and the EU (**Table 1**). The wines contained at least two of the target fungicides; most of them (70%) contained four or more compounds, and 50% contained 5-7 compounds. At least one anti-mildew agent was found in 100% of the samples, with 67% of the wines containing 2-3 anti-mildew compounds, being the most frequent (86%) combinations being (R)-metalaxyl and dimethomorph. All samples with the exception of two (one Treixadura and one Godello wines) contained one anti-botrytis product, and 12 out of 21 samples (67%) were treated with 2-3 anti-botrytis products, the vast majority (95%) with cyprodinil, and 52% with iprodione. Myclobutanil was the only anti-oidium agent to be quantified in the wines (57% of samples). By grape variety, Albariño wines contained the higher number of fungicide compounds (3-7). The studied non-Albariño wines contained lower number of fungicides.

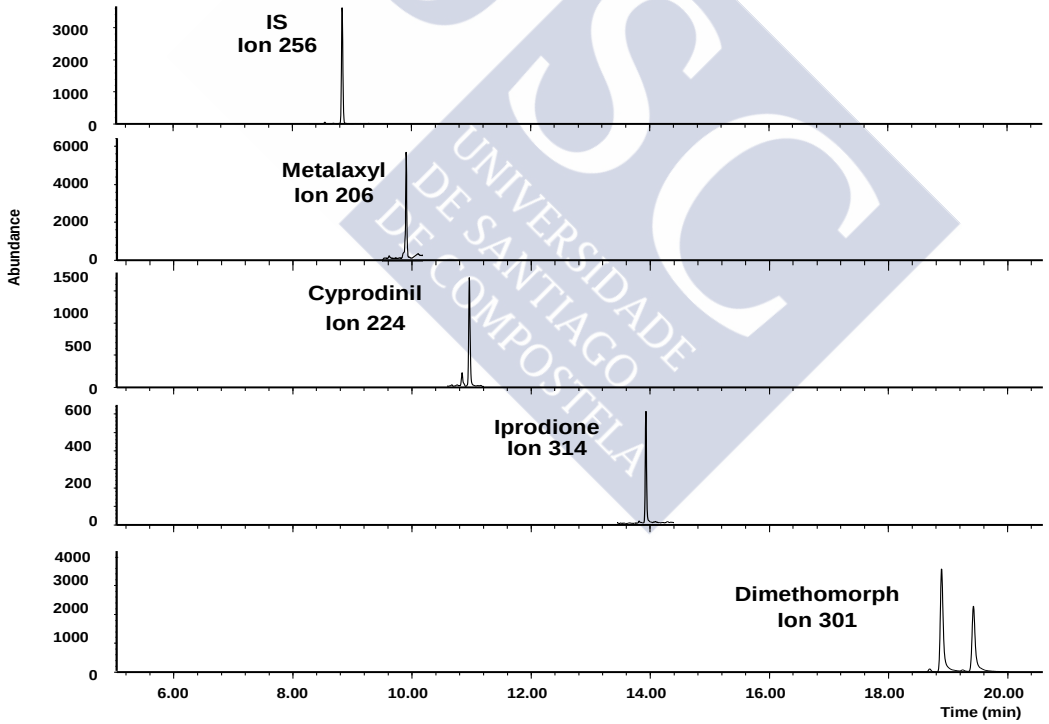

Figure 4. GC-MS (SIM) chromatogram of a non-spiked white wine sample (Loureira grape variety).

The total concentration of fungicides in the wine samples varied over a wide range from 1.34 to 696 ng mL^{-1}. Eighty percent of the samples contained >20 ng mL^{-1}. Higher concentrations were generally found in Albariño wines, with four samples containing ≥500 ng mL^{-1}, mainly due to the anti-mildiu dimethomorph. Important contributions also came from (R)-metalaxyl, iprodione, and, to a lesser extent, from cyprodinil. These results are in agreement with those found by other authors in wines. Metalaxyl was found in 5 of 7 white Galician wines at concentrations of 4-50 $ngmL^{-1}$ by Fontana et *al*. [6], who also found cyprodinyl, procymidone and iprodione in most of the wine samples at concentrations generally below 100 $ngmL^{-1}$; benalaxyl was present in only 1 of the 7 samples at 4 $ngmL^{-1}$, Pérez-Ortega et *al*. [4] found metalaxyl in 42% of commercial red wines from several Spanish regions at concentrations of 9-320 $ngmL^{-1}$, whereas dimetomorph appeared in only 20% of wines at concentrations generally lower than those found by us (2-9 ng mL^{-1}). In Galician white wines, Rial-Otero et *al*. [25] and González-Rodríguez et al [23] reported the presence of cyprodinil and benalaxyl at concentrations ranging from 1 to 100 ng mL^{-1}, which are in the same range of those found in the present work. **Fig. 4** shows the chromatogram obtained for a non-spiked wine sample (L1).

Table 6. Target fungicides found in white wine samples[a] (ng mL^{-1}), (RSD).

Compound	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	Tr1	Tr2	Tr3	To	Ca	BL	L1	L2	G1	G2	N[b]	Median conc.
Benalaxyl		0.59 (2.4)		<LOQ										0.91 (3.3)								3	0.75
Cyprodinil	32.2 (7.7)	5.88 (0.69)	0.21 (0.62)	22.0 (0.6)	0.48 (9.2)	0.31 (2.1)	0.19 (4.1)	0.19 (4.5)	0.61 (8.2)	0.83 (9.8)	0.28 (1.8)	<LOQ	3.98 (0.56)	10.7 (4.8)	4.10 (3.08)	0.25 (0.56)	3.85 (1.2)	0.45 (5.2)	0.13 (11)	0.99 (11)		20	0.61
Dimethomorph (E)	300 (6.1)	35.6 (1.9)	302 (2.1)	75.0 (2.1)	116 (6.8)	0.33 (4.4)		122 (7.2)	2.23 (9.9)	170 (4.3)	0.19 (16)		26.5 (10)	10.8 (7.6)	23.7 (12.9)	9.85 (8.4)	0.98 (9.8)	25.7 (12)	0.22 (17)		2.61 (13)	18	24.7
Dimethomorph (Z)	212 (6.4)	27.1 (0.7)	219 (2.2)	62.7 (3.6)	104 (6.1)	0.23 (9.0)		131 (6.4)	1.80 (2.4)	114 (4.6)	0.22 (9.7)		21.9 (12)	4.18 (4.9)	18.5 (10.6)	7.05 (10)	0.76 (14)	21.2 (11)	<LOQ		2.14 (10)	18	21.2
Iprodione		43.2 (11)	27.8 (5.4)	11.0 (8.4)			17.8 (5.1)	362 (3.8)	2.34 (10)	166 (6.0)				3.16 (5.3)		20.6 (20)		8.95 (10)		16.3 (10)		11	17.8
Kresoxim-methyl					<LOQ																	1	
(R)-Metalaxyl	0.46 (8.9)	45.1 (0.2)	6.43 (0.52)	77.1 (1.5)	0.44 (5.42)	1.53 (4.0)	31.6 (3.8)	79.4 (2.9)	19.8 (8.9)	47.1 (10)		54.8 (3.9)		24.9 (3.9)		6.12 (0.21)	12.0 (6.9)	6.12 (7.7)	1.26 (3.7)	23.0 (9.1)		17	19.8
Myclobutanil		1.80 (5.7)	0.49 (0.78)	0.84 (2.1)		3.64 (8.1)		1.85 (7.5)	1.94 (0.37)	2.94 (14)	0.65 (3.2)			1.03 (7.2)				<LOQ	2.89 (7.0)	0.54 (7.3)		12	1.80
Procymidone		1.17 (2.2)		3.79 (0.73)		0.37 (6.2)											8.72 (7.1)		0.66 (6.7)			5	1.17
Total conc.	545	170	556	252	221	6.41	49.6	696	28.7	501	1.34	54.8	52.4	55.7	46.3	43.9	26.3	62.4	5.16	40.8	4.75		52.4

[a] Grape variety keys. A: Albariño; Tr: Treixadura; To: Torrontes; Ca: Caiño; BL: Branca lexitima; L: Loureira; G. Godello.

[b] Number of wine samples with the target fungicides detected or quantified.

CONCLUSIONS

In the present work, USAEME is proposed as an efficient, rapid and low-cost sample preparation technique for the GC-MS analysis of fungicides in white wines under the optimized experimental conditions established after a multivariate study of the USAEME process. Good recoveries (70-115%) were obtained for all compounds. The precision of the method was satisfactory (RSD<12%) and quantification limits at the sub-nanogram per millilitre level were obtained (0.06-0.39 ng mL^{-1}). The validated method was applied to the analysis of white wines elaborated with Galician autochthonous grape varieties. The presence of the studied fungicides was confirmed in all the samples with a minimum of 2 and a maximum of 7 compounds per sample at total fungicide concentrations ranging from 1 to 700 ng mL^{-1}.

Acknowledgements

This research was supported by FEDER funds, and the 09TAL012209PR project and the Research Groups Consolidation Program (CN 2012/299), both funded by Xunta de Galicia government.

References

[1] Regulation (EC) No 396/2005 of the European Parliament and the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin, and amending Council Directive 91/414/EEC (Off. J.L70, 16 March 2005, pp. 1-16).

[2] Résidus de Pesticides dans les vins. In.Que Choisir? Union Française de Consommateurs, October 2013.

[3] A. Economou, H. Botitsi, S. Antoniou, D. Tsipi, J. Chromatogr. A, 1216 (2009) 5867.

[4] P. Perez-Ortega, B. Gilbert-Lopez, J. F. Garcia-Reyes, N. Ramos-Martos, A. Molina-Diaz, J. Chromatogr. A, 1249 (2012), 32-40.

[5] L. Lagunas-Allue, J. Sanz-Asensio, M.T. Martinez-Soria, J.AOAC Int., 95 (2012), 1511-1519.

[6] A. R. Fontana, I. Rodriguez, M. Ramil, J. C. Altamirano, R. Cela, J. Chromatogr. A, 1218 (2011), 2165.

[7] S. Walorczyk, D. Drozdzynski, B. Gnusowski, Talanta, 85 (2011), 1856-1870.

[8] T. Rodriguez-Cabo, I. Rodriguez, M. Ramil, R. Cela, J. Chromatogr. A, 1218 (2011), 6603-6611.

[9] L. M. Ravelo-Perez, J. Hernandez-Borges, T. M. Borges-Miquel, M. A. Rodriguez-Delgado, Food Chem., 111 (2008), 764-770.

[10] A. Bordagaray, R. Garcia-Arrona, E. Millan, Food Anal. Methods, 4 (2011), 293-299.

[11] C. Almeida, J.M.F. Nogueira, J. Chromatogr. A, 1265 (2012), 7-16.

[12] M. Moeder, C. Bauer, P. Popp, M. van Pinxteren, T. Reemtsma, Anal. Bioanal. Chem., 403 (2012), 1731-1741.

[13] A. R. Fontana, I. Rodriguez, M. Ramil, J. C. Altamirano, R. Cela, Anal. Bioanal. Chem., 401 (2011), 767-775.

[14] J. Regueiro, M. Llompart, C. Garcia-Jares, J. C. Garcia-Monteagudo, R. Cela, J. Chromatogr. A, 1190 (2008), 27-38.

[15] V. Andruch, M. Burdel, L. Kocurova, J. Sandrejova, I. S. Balogh, TrAC, Trends Anal.Chem. 49 (2013), 1-19.

[16] A. Szreniawa-Sztajnert, B. Zabiegala, J. Namiesnik, TrAC, Trends Anal. Chem. 49 (2013), 45-54.

[17] C. Pizarro, C. Saenz-Gonzalez, N. Perez-del-Notario, J. M. Gonzalez-Sainz, J. Chromatogr. A, 1229 (2012), 63-71.

[18] V. P. Jofre, M. V. Assof, M. L. Fanzone, H. C. Goicoechea, L. D. Martinez, M. F. Silva, Anal. Chim. Acta, 683 (2010), 126-135.

[19] X. You, S. Wang, F. Liu, K. Shi, J. Chromatogr. A, 1300 (2013), 64-69.

[20] J. Regueiro, M. Llompart, E. Psillakis, J. C. Garcia-Monteagudo, C. Garcia-Jares, Talanta, 79 (2009), 1387-1397.

[21] International Maximum Residue Limit Database. Available at: http://www.fas.usda.gov/ht p/MRL.asp (accessed September 2013)

[22] E. Becerril-Bravo, J. Pablo Lamas, L. Sanchez-Prado, M. Lores, C. Garcia-Jares, B. Jimenez, M. Llompart, Chemosphere, 81, (2010), 1378-1385.

[23] R. M. Gonzalez-Rodriguez, B. Cancho-Grande, J. Simal-Gandara, J. Chromatogr. A, 1216 (2009), 6033-6042.

[24] S. Wang, L. Ren, Y. Xu, F. Liu, Microchim. Acta, 173 (2011), 453-457.

[25] R. Rial Otero, C. Yague Ruiz, B. Cancho Grande, J. Simal Gandara, J. Chromatogr. A, 942 (2002), 41-52

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2.2. DETERMINATION OF FUNGICIDES IN WHITE GRAPE BAGASSE BY PRESSURIZED LIQUID EXTRACTION AND GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

Journal of Chromatography A (Ed. Elsevier, Netherlands)
ISSN: 0021-9673

***J. Chromatogr. A., 1343 (2014) 18-25*. doi: 10.1016/j.chroma.2014.03.057**

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DETERMINATION OF FUNGICIDES IN WHITE GRAPE BAGASSE BY PRESSURIZED LIQUID EXTRACTION AND GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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ABSTRACT

Ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE) followed by gas chromatography-triple quadrupole-mass spectrometry (GC TQ-MS) were used for the rapid determination of 11 fungicides (metalaxyl, cyprodinil, procymidone, iprovalicarb, myclobutanyl, kresoxim-methyl, benalaxyl, fenhexamide, tebuconazole, iprodione and dimethomorph) in white grape bagasse. Extractions were optimized on real non-spiked samples by means of experimental design and the optimal conditions were selected to accomplish method validation. PLE procedure showed much higher efficiency than UAE for the target fungicides. Under the selected extraction conditions PLE showed satisfactory linearity, repeatability and reproducibility. Recoveries for the majority of studied fungicides were higher than 80% with relative standard deviations (RSD) lower than 12%. Limits of detection (LODs) for GC TQ-MS were very low, at the sub ng g^{-1} for the majority of the target fungicides, well below European maximum residue limits (MRLs) for wine and table grapes, and vine leaves. Eighteen white grape bagasse samples were analyzed and nine out of eleven targets were detected in the samples. Seven of them were detected in more than 50% of the samples and most samples contained at least four of the target analytes. The most frequently found compounds were tebuconazole and dimethomorph with concentrations between 1.6-130 and 2.0-1788 ng g^{-1} respectively. Some samples showed high levels of many of the studied fungicides (high ng g^{-1}, even µg g^{-1} for cyprodinil, fenhexamide, iprodione and dimethomorph), but all of them below of the European maximum residue limits (MRLs) for wine grapes.

Keywords: fungicides; bagasse; ultrasound-assisted extraction; pressurized liquid extraction; GC-MS; GC-TQ-MS.

1. INTRODUCTION

There is an increasing interest regarding health and safety aspects associated with the use of pesticides and the presence of their residues in food and drinks. Pesticides are used to control pests in vegetables, fruits or cereal grains among others [1]. Fungicides are a class of pesticides widespread used in viticulture to avoid fungi infection of *Vitis* plants, being mainly used for treating grey rot (*Botrytis cinerea*), mildew (*Plasmopara viticola*) and oidium (*Uncinula necator*). Their use has brought many benefits with respect to enhanced quality of produced crops, but there are concerns about the presence of their residues in crops, which may pose a health hazard to the consumers. In addition, several studies have shown that some fungicides and their degradation products can be found in musts and some of them are frequently found at low concentration levels in the final commercial wine [2-5].

Bagasse (also called marc) is the residue left behind after the juice has been removed for bunch of grapes during winemaking. There is an increasing interest, supported by environmental and economic reasons, to recover and exploit these wastes from the food industry, because such residues can be used as a source of natural bioactive compounds, which could in turn be used in pharmaceutical, cosmetics or back in the food industry. Therefore, the levels of fungicides in grape bagasse must be controlled in order to avoid environmental pollution and human exposure to these compounds [6]. The European Community establishes the maximum residue limits (MRLs) for different fungicides in wine and table grapes as well as in vine leaves through EC Regulation 396/2005 and their subsequent amendments [7], but no harmonized MRLs have been laid down in the European Union for pesticides in bagasse or wine. European MRLs set for the target fungicides in wine and table grapes, and in vine leaves are shown in **Table 1**.

Table 1. European maximum residue limits (MRLs) for wine and table grapes and vine leaves [7].

Fungicides	Wine grapes ($mg\ kg^{-1}$)	Table grapes ($mg\ kg^{-1}$)	Vine leaves ($mg\ kg^{-1}$)
Metalaxyl	1	2	0.05
Cyprodinil	5	5	0.05
Procymidone	0.01	0.01	0.01
Iprovalicarb	2	2	0.05
Myclobutanyl	1	1	0.02
Kresoxim- Methyl	1	1	0.05
Benalaxyl	0.3	0.3	0.05
Fenhexamide	5	5	0.05
Tebuconazole	2	2	0.05
Iprodione	10	10	0.02
Dimethomorph	3	3	0.01

Nevertheless, a look at the scientific literature evidences the lack of studies devoted to the development of methodology for the determination of fungicides in bagasse samples whereas in grapes, other fruits and vegetables, environmental friendly procedures including microwave assisted extraction (MAE) [8], QuEChERS [9-12], solid phase micro-extraction (SPME) [13,14] or matrix-solid phase dispersion (MSPD) [15-17] are substituting traditional methodologies like Soxhlet extraction [18,19]. Pressurized liquid extraction (PLE) was also employed to determine various chemical classes of fungicides in different matrices such as mushroom compost [20], vineyard and agricultural soils [21,22], green leafy vegetables [23] or green tea [24].

The analytical methods for detecting and quantifying fungicides in different fruits are generally based on liquid chromatography (LC) or gas chromatography (GC). The decision to use either LC or GC is based on to physico-chemical properties of the target analytes. The on-line coupling of efficient liquid chromatography or gas chromatography separation with mass spectrometry detection (LC-MS or GC-MS) has became an accepted technique for performing regulatory monitoring. GC-MS is an advantageous and powerful technique for the determination of (semi)volatile and low polarity fungicides in vegetable samples and LC-MS is best choice for substances with low volatibility and/or thermal instability [1]. Liquid or gas chromatography in combination with tandem mass spectrometry (MS-MS) is a valuable approach that improves selectivity and analyte sensitivity, minimizing most of the matrix interferences. In this way, triple quadrupole (TQ) working under MS/MS mode can achieve lower limits of detection than simple quadrupole GC SQ-MS or LC TQ-MS and increasing the selectivity and specificity of the method. LC TQ-MS was successfully employed to determinate fungicides in cereals, vegetables and fruits [9], grapes [1] or food (lettuce, tomato, apple and grapes) [11] and although there are few references of GC TQ-MS to determine fungicides in vegetables or fruit samples, this technique was recently used to analyze more than 140 pesticides in vegetables [26,27].

The aim of this study was to develop and validate a method to analyze 11 fungicides from different chemical classes (metalaxyl, cyprodinil, procimidone, iprovalicarb, myclobutanyl, kresoxim-methyl, benalaxyl, fenhexamide, tebuconazole, iprodione and dimethomorph) in white grape bagasse, based on pressurized liquid extraction-gas chromatography-triple quadrupole-mass spectrometry. This study also aimed at comparing GC TQ-MS with GC SQ-MS to determine whether the use of the former technique is an improvement for the detection of the target fungicides. Finally, the validated method was used to identify and quantify the studied fungicides in real white grape bagasse. To the best of our knowledge, this is the first time that PLE-GC TQ-MS is applied to the analysis of fungicides in bagasse samples.

2. MATERIALS AND METHODS

2.1. Chemicals, materials and samples

The studied compounds, their chemical names, CAS numbers, suppliers and purity are summarized in **Table 2**.

Table 2. Target compounds: purity, suppliers, CAS numbers, and GC-MS parameters.

Fungicides	Purity (%)[a]	CAS	Retention time (min)	**Quantification** and identification ions[b]
Metalaxyl	99.5	70630-17-0	9.67	192.1 (42), 160.1 (57), **206.1** (100)
Cyprodinil	97.5	32809-16-8	10.77	210.1 (10), **224.0** (100), 225.1 (61)
Procimidone	99.5	88671-89-0	11.07	96.0 (203), **283** (100), 285 (65)
Iprovalicarb	99	140923-17-7	11.74/11.90	116.1 (99), 119.0 (71), **134.0** (100)
Myclobutanyl	97.5	143390-89-0	11.95	150.0 (51), **179.0** (100), 245.0 (45)
Kresoxim-Methyl	98	71626-11-4	11.89	116 (214), **131** (100), 206.1 (92)
Benalaxyl	98	121552-61-2	12.94	91.0 (45), **148.1** (100), 206.1 (26)
Fenhexamide	99	126833-17-8	13.28	55.0 (40), **97.0** (100), 177.0 (34)
Tebuconazole	99	107534-96-3	13.47	83.0 (84), 125 (145), **250.0** (100)
Iprodione	97.5	36734-19-7	13.80	186.9 (51), 244.8 (20), **313.9** (100)
Dimethomorph	98	110488-70-5	18.87/19.43	165.0 (32), **301.0** (100), 387.1 (29)

[a]Dr. Ehrenstorfer (Ausburg, Germany).
[b]Numbers in brackets are the relative ion abundances, %.

Acetone, ethyl acetate and n-hexane were provided by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Methanol was provided by Merk (Darmstadt, Germany). Sand (200-300 µm) was purchased from Scharlau (Barcelona, Spain) and sodium chloride (NaCl) was provided by Prolabo (Leuven, Belgium).

Individual stock solutions of each compound were preparated in methanol or ethyl acetate. Further dilutions and mixtures were prepared in acetone (sample fortification solutions) and hexane/acetone (calibration standards). All solutions were stored in amber glass vials at -20°C. All solvents and reagents were of analytical grade.

Bagasse samples were selected among five different varieties of white grape of Galicia (Albariño, Caiño, Loureira, Treixadura and Godello). Samples were dried at 60°C for 24 hours before use, and subsequently were crushed in a conventional coffee grinder and pulverized in a porcelain mortar using a porcelain pestle until a homogeneous mixture was obtained (ca. 5 min).

2.2. UAE procedure

Ultrasound-assisted extraction (UAE) was carried out using an ultrasonic cleaning bath with a working frequency of 50 kHz and 110 W of power (Ultrasound Med-II, J.P. Selecta, Barcelona, Spain). 0.5 g of bagasse were mixed with 5 mL of appropriate extraction solvent (ethyl acetate, hexane:acetone (1:1 v/v), methanol or hexane) in a 10 mL vial that was placed in the ultrasound bath. The mixture bagasse sample-solvent was sonicated (15 min) at different temperatures (25 or 45°C) and with NaCl (0 or 20% w/v). Afterwards, the supernatants were filtered through 0.45-µm

PTFE microporous filters (25 mm diameter), evaporated under a gentle nitrogen stream and reconstituted in ethyl acetate. The extracts, diluted when necessary, were analyzed by GC-MS and GC TQ-MS.

2.3. PLE procedure

Extractions were performed on an ASE 150 (Dionex, Co., Sunyvale, CA, USA), equipped with 10 mL stainless steel cells and 60 mL collection vials. Two cellulose filters (Dionex) were placed at each end of the PLE cell. 0.5 g of sample were weighted and introduced into the cell, where previously 1 g of clean sand (200-300 µm mesh particle size, Scharlau, Barcelona, Spain) was placed. For the preparation of fortified samples, the sample bagasse was spiked with 10 µL of the corresponding acetone solution of the target compounds to get the desired final concentration. Finally, the dead volume of the cell was filled with sand. The cell was tightly closed and placed into PLE system. Extractions were performed by preheating the cell before filling with solvent (preheat method). The extraction pressure was 1500 psi, the flush volume was 60%, and the purge time 60 s. Hexane:acetone (1:1, v/v) was employed as extraction mixture. Three temperatures were studied (80, 100 and 120°C) at 5, 10 and 15 min. In all cases, the extracts were levelled to a final volume of 20 mL and were analyzed by GC-MS and GC TQ-MS.

2.4. GC-MS analysis

The GC–MS analysis was performed using an Agilent 7890A (GC)-Agilent 5975C inert MSD with triple axis detector and an Agilent 7693 autosampler from Agilent Technologies (Palo Alto, CA, USA). The temperatures of the transfer line, the quadrupole and the ion source were set at 290, 150 and 230°C, respectively. The system was operated by Agilent MSD ChemStation E.02.00.493 software.

Separation was carried out on a cross-linked 5%-phenyl polysililphenylen-siloxane TR-5 MS capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness) obtained from Thermo Scientific (Palo Alto, CA, USA). Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 mL min^{-1}. The GC oven temperature was programmed from 100°C (held 2 min) to 200°C at 20°C min^{-1}, to 260°C at 10°C min^{-1} and 20°C min^{-1} to 290°C. Pulsed splitless mode was used for injection (30 psi, held 1.2 min). After 1 min, the split valve was opened and the injector temperature was kept at 260°C. The injection volume was 1 µL. The mass spectra detector (MSD) operated in selected ion monitoring (SIM) mode, monitoring three ions per compound (**Table 2**). The electron multiplier was set at a nominal value of 1612 V.

2.5. GC TQ-MS analysis

The GC TQ-MS analysis was performed using a Thermo Trace 1310-Triple Quadrupole 8000 with autosampler IL 1310 from Thermo Scientific (San Jose, CA, USA). The temperatures of the

transfer line, and the ion source were set at 290 and 350°C, respectively. The system was operated by Xcalibur 2.2 and Trace Finder TM 3.0.

Separation was carried out on a TG-5 SILMS capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness) obtained from Thermo Scientific (San Jose, CA, USA). Helium (purity 99.999%) was employed as carrier gas at a constant column flow of 1.0 mL min^{-1}. The GC oven temperature was programmed from 100 °C (held 2 min) to 200°C at 20°C min^{-1}, to 260°C at 10°C min^{-1} and 20°C min^{-1} to 290°C. Splitless w/Surge mode was used for injection (200 kPa, held 1.2 min). After 1 min, the split valve was opened and the injector temperature was kept at 260°C. The injection volume was 1 µL. The mass spectra detector (MSD) operated in selected reaction monitoring acquisition mode (SRM), monitoring two transitions per compound (**Table 3**). The electron multiplier was set at a nominal value of 1567 V.

2.6. Statistical analysis

Basic and descriptive statistics, as well as experimental design analysis were performed using Statgraphics-Plus v5.1 (Manugistics, Rockville, MD, USA) as software package. An experimental design was applied for the optimization of the extraction method, to analyze the simultaneous effect of the experimental parameters affecting UAE and PLE.

3. RESULTS AND DISCUSSION

3.1 Optimization of the chromatographic conditions GC-MS and GC TQ-MS

The chromatographic conditions were optimized to achieve an efficient separation of the 11 target compounds (see conditions in the experimental section). For GC-MS analysis, the MS detector was operated in the SIM mode selecting three ions per compound and the GC TQ-MS/MS detector was operated in the SRM mode selecting two transitions per compound. **Tables 2 and 3**.

Table 3. GC TQ-MS retention time and selected transitions.

Fungicides	Retention time (min)	Precursor Ion[a]	Product Ion[a]	Collision Energy[a]
Metalaxyl	11.62	206.1	132.0	10
		234.1	174.1	10
Cyprodinil	12.91	224.1	208.1	20
		225.1	210.1	18
Procimidone	13.27	283.0	96.0	15
		283.0	255.0	10
Iprovalicarb	14.04	116.0	55.1	10
		116.0	98.0	15
Myclobutanyl	14.26	179.1	125.0	15
		179.1	152.1	15
Kresoxim-Methyl	14.20	206.1	131.1	15
		206.1	116.1	15
Benalaxyl	15.36	234.1	174.1	10
		266.1	148.1	10
Fenhexamide	15.78	177.0	113.1	10
		301.1	97.0	15
Tebuconazole	16.02	250.1	125.1	20
		252.1	127.1	20
Iprodione	16.58	314.0	245.0	15
		316.0	247.0	15
Dimethomorph	23.92/24.60	301.1	165.1	10
		387.1	301.1	12

[a]Underlined values are the quantification transitions.

3.2. Optimization of the extraction process

The GC-MS instrument was employed for the optimization of the extraction procedure.

3.2.1. Ultrasound assisted extraction

First efforts were focused in the development of an *"easy to implement"* low cost methodology based on the use of ultrasounds energy. Ultrasounds extraction employing an ultrasonic bath is a strategy affordable for any laboratory due to its low cost and simplicity of use. Most extraction optimization studies are carried out on spiked sample, implying that the real interaction of the sample with the analytes is not assessed. In the present study, a real non-spiked bagasse sample containing most target compounds was employed. The process was optimized by means of a multifactor experimental design 4×2^3. Three factors were included: the extraction solvent, the temperature, and the addition of NaCl. The first factor was studied at four levels, and so the performance of four solvents was tested: ethyl acetate, hexane/acetone (1:1, v/v), methanol and hexane. The other two factors were studied at two levels: 25 and 45°C for the temperature; and 0 and 20 % (w/v) for the NaCl. Other factors such as the amount of sample (0.5 g) and the extraction time (15 min) were maintained invariable. The results for the multifactor ANOVA study are shown in **Table 4**. As can be seen, the solvent was the most relevant factor being statistically significant for all analytes. The other factors, temperature and salt addition, were not significant. **Fig 1** shows the mean plot charts for the solvent. The use of methano

produces the lowest responses whereas hexane/acetone mixture provides maximum response. The mean plots for the other two factors are depicted in **Fig 2** for some representative compounds. As shown in the figure, better response is achieved at 25°C for iprovalicarb and without salt addition for tebuconazole. For the other compounds, those factors were no significant. The second order factors (interactions) were not significant in all cases.

Table 4. ANOVA study: F ratios and p values obtained for ultrasound-assisted extraction (UAE).

Fungicides	A: Solvent		B: Temperature (ºC)		C: NaCl (%)		AB		AC		BC	
	F	p	F	p	F	p	F	p	F	p	F	p
Metalaxyl	**14**	**0.02**	0.43	0.56	0.14	0.73	0.30	0.83	6.0	0.09	0.40	0.57
Cyprodinil	**56**	**0.004**	6.2	0.08	0.00	0.99	2.6	0.23	4.2	0.14	1.1	0.38
Iprovalicarb	**77**	**0.002**	**14**	**0.03**	2.0	0.25	5.4	0.10	6.8	0.07	2.8	0.19
Myclobutanyl	**9.0**	**0.04**	0.35	0.60	0.66	0.48	0.70	0.61	0.84	0.55	0.35	0.60
Benalaxyl	**54**	**0.004**	0.40	0.57	**12**	**0.04**	5.9	0.09	4.9	0.11	0.48	0.54
Fenhexamide	**138**	**0.001**	2.6	0.21	0.01	0.93	3.2	0.18	5.5	0.09	0.85	0.43
Tebuconazole	**262**	**0.000**	1.5	0.30	**17**	**0.03**	3.0	0.19	1.2	0.44	1.5	0.30
Iprodione	**17**	**0.02**	0.26	0.65	4.4	0.13	0.87	0.54	1.5	0.37	0.01	0.94
Dimethomorph	**48**	**0.005**	0.44	0.55	0.08	0.80	1.5	0.37	3.4	0.17	1.5	0.31

p<0.05 denotes statistical significance (indicated in bold)

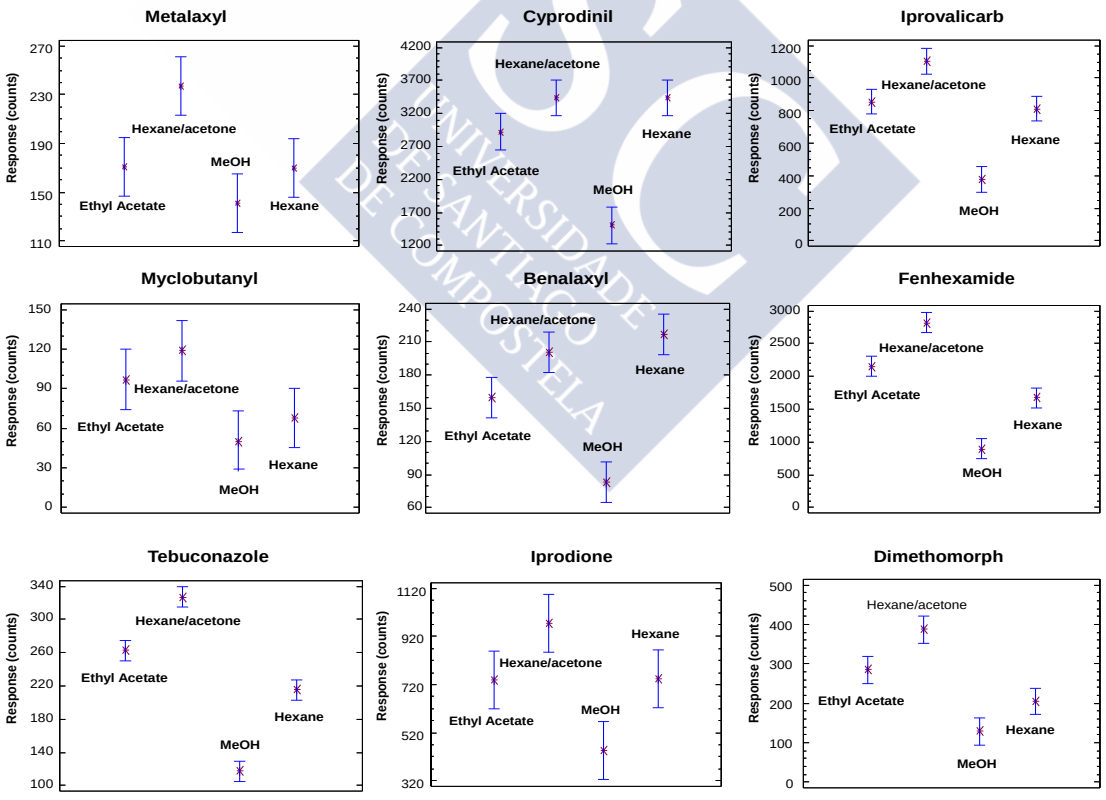


Fig. 1. UAE mean plot charts for the solvent

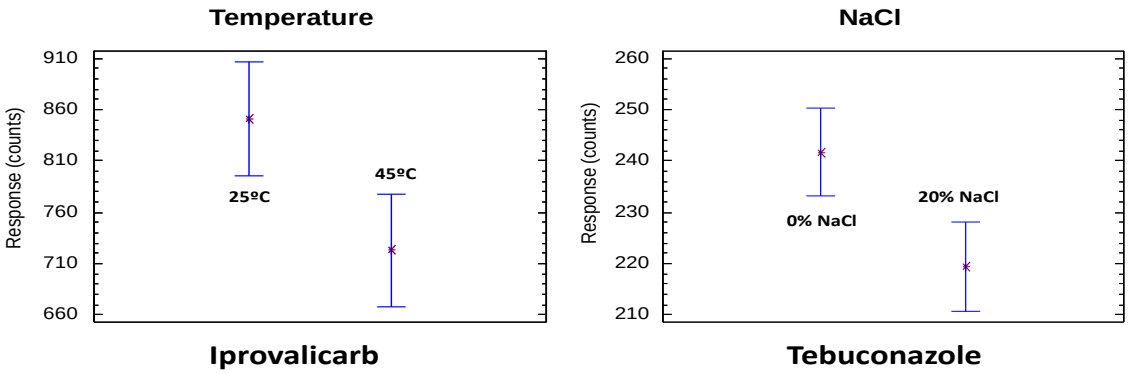


Fig. 2. UAE mean plot charts for temperature (iprovalicarb) and NaCl addition (tebuconazole)

3.2.2. Pressurized liquid extraction

Under the optimal conditions, UAE was compared with PLE for the same real sample. PLE extractions were performed at 80°C for 15 min. Results are summarized in **Fig 3**. Unexpectedly, the responses were clearly lower for UAE extraction and thus, we decided to continue the study using PLE. In these experiments, the sample size was 0.5 g and the extraction solvent hexane:acetone (1:1 v/v) since it was the most appropriate according to the previous study.

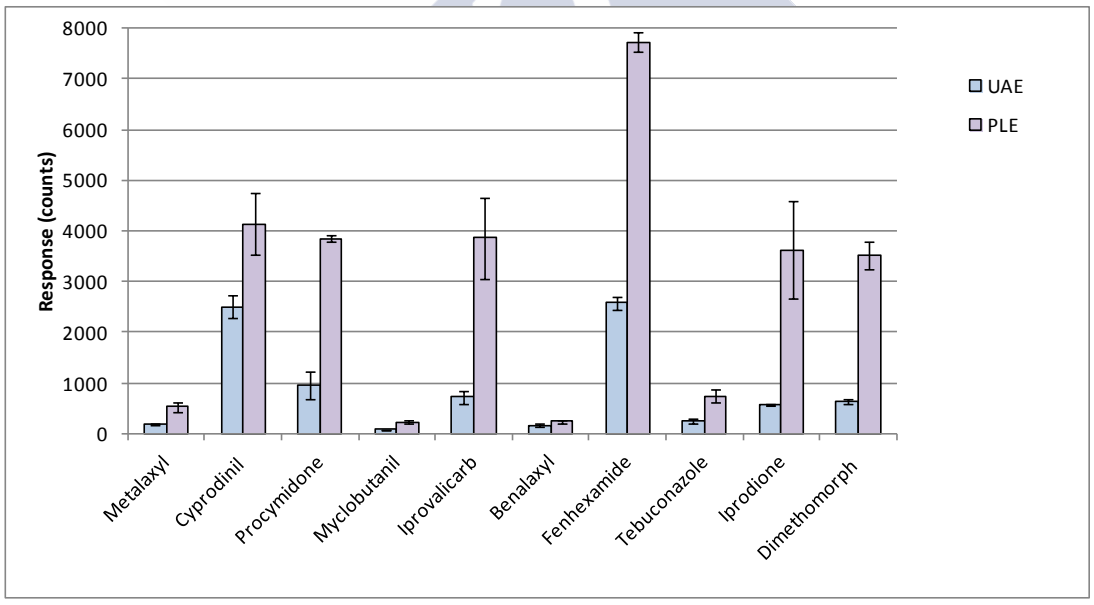
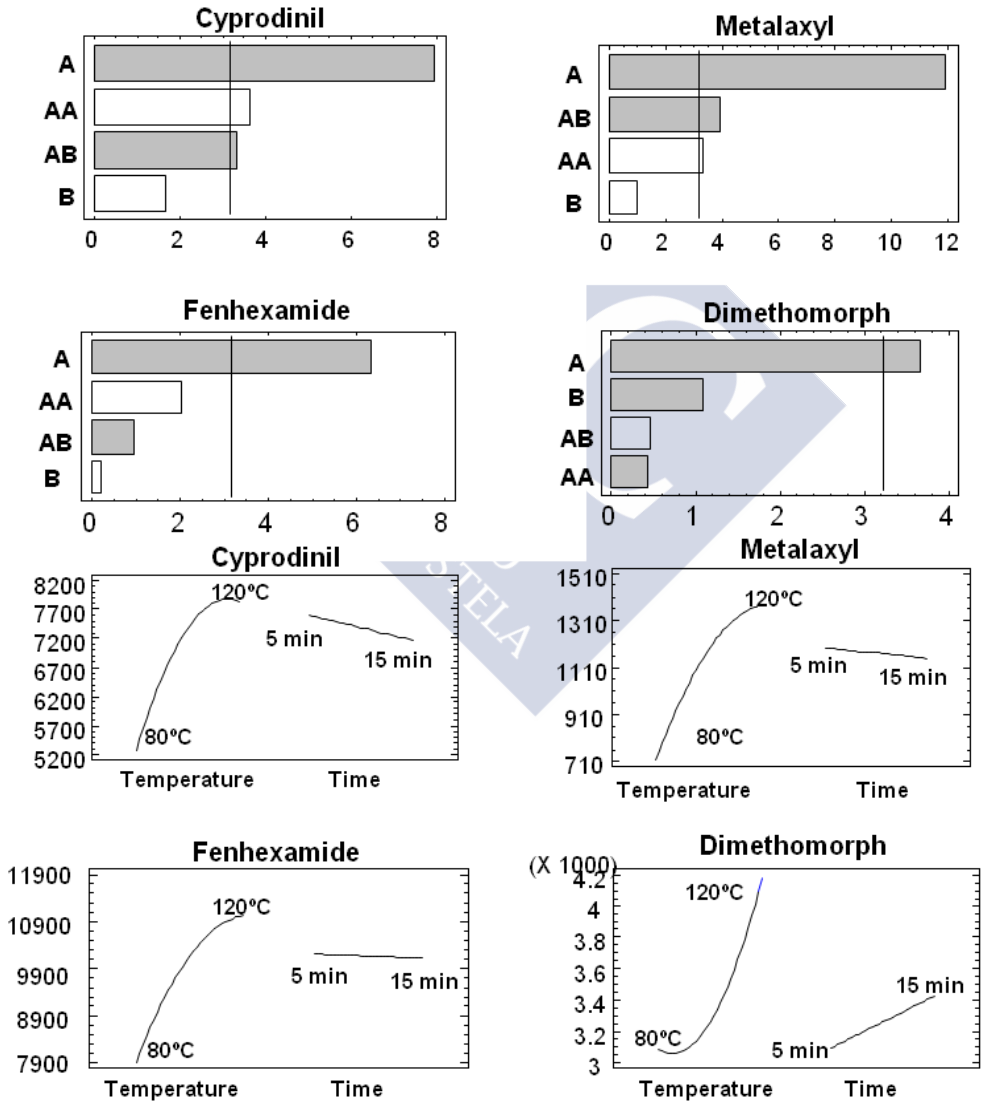


Fig. 3. Comparison between responses obtained by UAE and PLE.

Other two parameters which can drastically affect extraction, the PLE temperature (A) and time (B), were studied at three levels: 80, 100 and 120 °C and 5, 10 and 15 min respectively, and optimized by means of an experimental design 2^3. Once again, the study was performed using a real non-spiked bagasse sample. The outcomes of the experimental design can be simply interpreted by visualizing several intuitive software tools provided by Statgraphics. In the Pareto

charts (**Fig 4**), the standardized effects are plotted in decreasing order of absolute magnitude, thus making easier to see which ones are the most important factors and interactions. In addition, the line drawn on the chart indicates whether an effect is statistically significant at a specified significance level (in this case, 95%). Main effect plots (**Fig 4**) show how the response varies when each factor is changed from its low level to its high level, while all others factors are held at the center of the experimental domain. In **Fig 4**, the pareto charts and the main effect plots for the analytes showing significant effects are included. Temperature (A) was significant for four of the ten target analytes present in the sample. The quadratic term AA was also significant for metalaxyl and cyprodinil showing a maximum around 120°C. On the other hand, the time (B) was not significant for any of the compounds. Therefore, 5 min and 120°C were the experimental conditions selected.

Fig. 4. PLE pareto charts and main effect plots for the analytes showing significant effects.



3.3. Method performance

The GC-MS and GC TQ-MS method performance parameters are summarized in **Table 5**. Regarding the instrumental linearity, methods exhibited a direct proportional relationship between the amount of each analyte and the chromatographic response. Calibration standards in hexane:acetone (1:1 v/v), were prepared covering a concentration range from 2 to 1000 ng mL^{-1}. Correlation coefficients R≥0.993 for GC-MS analysis and R≥0.998 for GC TQ-MS analysis were obtained. Method precision was studied within a day (n=5) and among days (n=9) at two concentration levels (20 ng mL^{-1} and 200 ng mL^{-1}). For GC-MS analysis, RSD values ranged from 0.02 to 11% (intraday precision), and from 3.2 to 14% (interday precision). For GC TQ-MS, RSD values ranged from 0.59 to 12% (intraday precision), and from 3.3 to 13% (interday precision). Instrumental detection limits (IDLs) were calculated as the concentration giving a signal-to-noise ratio of three (S/N = 3). The obtained values were below 1 ng mL^{-1} in GC-MS analysis for the majority of the studied fungicides. For GC TQ-MS, they were much lower than for GC-MS, namely below 0.05 ng mL^{-1} (**Table 5**).

Table 5. Method quality parameters.

	GC-MS (SIM)		GC TQ-MS (SRM)		Recoveries, % (RSD)				Method detection and quantification limits			
					GC-MS (SIM)		GC TQ-MS (SRM)		GC-MS (SIM)		GC TQ-MS (SRM)	
Fungicides	Correlation coefficient (R	IDL (ng mL^{-1})	Correlation coefficient (R)	IDL (ng mL^{-1})	100 ng g^{-1}	1000 ng g^{-1}	100 ng g^{-1}	1000 ng g^{-1}	LOD (ng g^{-1})	LOQ (ng g^{-1})	LOD (ng g^{-1})	LOQ (ng g^{-1})
Metalaxyl	0.9996	0.23	0.9995	0.013	81 (5.6)	102 (10)	94 (8.0)	100 (5.4)	9.20	30.4	0.52	1.72
Cyprodinil	0.9998	0.12	0.9997	0.011	99 (6.3)	107 (9.7)	100 (9.9)	96 (5.1)	4.80	15.8	0.44	1.45
Procymidone	0.9998	0.61	0.9988	0.007	94 (4.1)	99 (12)	99 (8.6)	97 (5.4)	24.4	80.5	0.28	0.92
Iprovalicarb	0.9996	0.74	0.9997	0.049	91 (5.8)	112 (10)	111 (7.1)	118 (8.3)	29.6	97.7	1.96	6.47
Myclobutanyl	0.9995	0.48	0.9997	0.017	91 (6.7)	111 (6.7)	110 (11)	114 (6.1)	19.2	63.4	0.68	2.24
Kresoxim- Methyl	0.9995	0.71	0.9997	0.004	118 (7.7)	114 (12)	108 (8.8)	97 (5.7)	28.4	93.7	0.16	0.52
Benalaxyl	0.9996	0.64	0.9997	0.004	108 (11)	111 (9.1)	116 (12)	105 (12)	25.6	84.5	0.16	0.52
Fenhexamide	0.9930	28.0	0.9981	0.033	---	---	119 (5.9)	111 (2.3)	1120	3696	1.32	4.36
Tebuconazole	0.9989	0.57	0.9999	0.011	105 (7.6)	---	111 (9.6)	120 (5.8)	22.8	75.2	0.44	1.45
Iprodione	0.9992	6.82	0.9980	0.010	---	93 (16)	---	107 (9.5)	273	900	0.40	1.32
Dimethomorph	0.9993	0.73	0.9996	0.015	100 (12)	115 (9.9)	104 (7.9)	104 (7.3)	29.2	96.4	0.60	1.98

Method quality parameters were evaluated using real bagasse samples and they are shown in **Table 5**, as well. Recovery studies were carried out by applying the optimized method to the extraction of a real sample, spiked at 100 ng g^{-1}, and 1000 ng g^{-1}. Previous analyses of the samples showed the presence of some of the target compounds and these initial concentrations were taken into account to calculate the recoveries. As can be seen in **Table 5**, recoveries were between 81-120 % in all cases for GC-MS and GC TQ-MS. These recoveries can be considered quantitative and no

matrix effects were observed. Therefore, quantification by external calibration can be effectively employed. The absence of matrix effect could be attributed to the fact that the proposed method does not require concentration. 0.5 g of sample is extracted with 20 mL of solvent and the extract is directly analyzed. On the contrary, most trace organic analytical methods very often require a drastic concentration step to achieve the desired or required LODs. In those cases, matrix effects often become a great problem. Precision was also evaluated and RSD values were generally lower than 12 % for all fungicides. Limits of detection (LODs) and quantification (LOQs) were calculated as the concentration giving a signal-to-noise ratio of three (S/N)=3 and ten (S/N)=10, respectively. For GC TQ-MS, LODs values were below 1 ng g^{-1} and up to two orders of magnitude lower (even three orders for fenhexamide) than those obtained by GC-MS (see **Table 5**). Besides LODs and LOQs were several orders of magnitude lower than the European MRLs (**Table 1**) for wine and table grapes and vine leaves. In any case, we can conclude that the proposed method is highly sensitive, especially when TQ-MS detection is performed. It is important to emphasize that the PLE extract (20 mL) is directly analyzed without concentration and so if necessary these limits could be even improved by concentrating the PLE extract.

GC-MS and GC TQ-MS showed similar linearity, repeatability and reproducibility. Nevertheless GC TQ-MS offered lower IDLs and LODs (about two orders of magnitude) improving selectivity and sensibility which is a great advantage to detect and quantify trace levels in real samples.

3.4. Application to real samples

The validated method was applied to the analysis of 18 real white grape bagasse samples including five Galician varieties: Albariño (Alb), Caiño (Cai), Loureira (Lou), Treixadura (Tre) and Godello (God). Results are shown in **Table 6**. The target fungicides were detected in all of samples. Tebuconazole and dimethomorph were the most abundant (found in 17 and 16 samples respectively). Fenhexamide and myclobutanyl were found in 72 and 67% of the samples respectively. Metalaxyl, cyprodinil and iprodione were also detected in 9 of the 18 studied samples. Iprodione levels were quite high (6021 and 8800 ng g^{-1}) but they were below the European maximum residue limits (MRLs) for wine grapes (the highest MRL among our target compounds). Iprovalicarb and benalaxyl were detected in 4 and 13 samples respectively. Procymidone and kresoxim-methyl were not found. In general, Godello variety presented fewer fungicides (between 1 and 4) compared with the other 4 varieties. Fungicide concentration in all white grape bagasse samples were lower than the European maximum residue limits (MRLs) for wine grapes, excluding benalaxyl in a Treixadura sample (375 ng g^{-1}).

Table 6. Analysis of white grape bagasse samples (ng g^{-1})

Fungicides	Metalaxyl	Cyprodinil	Iprovalicarb	Myclobutanyl	Benalaxyl	Fenhexamide	Tebuconazole	Iprodione	Dimethomorph
Samples									
Alb_01	206			203		100	141		405
Alb_02		1007	390			4.14	5.85		318
Alb_03	572	155	873	110	37.7	1427	130	6021	1698
Alb_04				76.9			1.56		2.65
Alb_05	239			13.0		25.0	9.14		8.69
Alb_06	899	12.5		167		12.8	5.37	10.9	13.7
Alb_07				54.4		143.7	39.4	3.19	26.1
Cai_01	658	81.2		350.6		14.0	2.39	15.8	235
Cai_02		45.9	1001	506		16.1	15.3	6.30	67.0
Cai_03		261					1.50		2.74
Lou_01	87.7	3858		108		5.29	5.39	85.9	12.3
Lou_02						11.3	2.28		2.58
Tre_01	514					44.3	1.64	52.4	
Tre_02	34.8	1049	570	51.5	375		5.54	8801	
Tre_03	898	9.42		9.32		132	32.7	53.3	41.4
God_01				63.1	15.6		11.3		2.03
God_02						10.7	4.05		13.9
God_03									27.2

4. CONCLUSIONS

PLE has been successfully applied to the determination of 11 fungicides in white grape bagasse. To our knowledge, this study constitutes the first application of PLE-GC TQ-MS to the analysis of these compounds in bagasse samples. The most important parameters involved in the extraction were optimized using a multifactorial experimental design in real bagasse samples. Under optimized conditions, fungicides were extracted with hexane: acetone (1:1 v/v) for 5 min at 120ºC. Method accuracy and precision were satisfactory, showing mean recovery values higher than 80% and RSD was generally below 12%. We also compared GC-MS and GC TQ-MS techniques, the latter showing better IDLs and LODs than GC-MS analysis. In most cases, this difference was about two orders of magnitude, which undoubtedly provides an advantage for detecting trace levels of fungicides in real bagasse samples.

Acknowledgements

This research was supported by FEDER funds and project CN 2012/299 (Consolidated Research Groups Program, Xunta de Galicia, Spain).

References

[1] C.A. Damalas, I.G. Eleftherohorinos, Int. J. Environ. Res. Public Health, 8 (2011) 1402.

[2] R.C. Calhelha, J.V. Andrade, I.C. Ferreira, L.M. Estevinho, Food Microbiol., 23 (2006) 393.

[3] Pesticide Action Network, Europe (PAN). Available at: http://www.pan-europe.info/Resources/ Briefings/Message_in_a_bottle (accessed 21.11.13).

[4] R.M. González-Rodriguez, B. Cancho-Grande, J. Simal-Gandara, J. Chromatogr. A., 1216 (2009) 6033.

[5] S. Romero, C. Domingo, R. Franquet, J. Garcia, Le Bulletin de l'O.I.V, 2007, pp.241.

[6] M. Lores, M. Iglesias-Estevez, M. Alvarez-Casas, M. Llompart, C. Garcia-Jares, Recursos Rurais, 8, IBADER, Santiago de Compostela, Spain, 2012, pp.39.

[7] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on Maximum Residue Levels of Pesticides in or on Food and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC L70/01. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:070:0016:en:PDF (accessed 25.11.13).

[8] L. Lagunas-Allue, J. Sanz-Asensio, M.T. Martinez-Soria, Anal. Methods, 3 (2011) 2881.

[9] F. Dong, X. Chen, X. Liu, J. Xu, Y. Li, W. Shan, Y. Zheng, J. Chromatogr. A. 1262 (2012) 98.

[10] A.E. Afify, M.A. Mohamed, H.A. El-Gammal, E.R. Attallah, Int. J. Food. Agric. Environ. 8 (2010) 602.

[11] S.C.N. Queiroz, V.L. Ferracini, M.A. Rosa, Quimica Nova, 35 (2012) 185.

[12] X. Liu, X. Wang, J. Xu, F. Dong, W. Song, Y. Zheng, Biomed. Chromatogr., 25 (2011) 1081.

[13] A. Bordagaray, R. Garcia-Arrona, E. Millan, Anal. Methods. 5 (2013) 2565.

[14] E.A.S. Silva, V. Lopez-Avila, J. Pawliszyn, J. Chromatogr. A. 1313 (2013) 139.

[15] M.G. Silva, A. Aquino, H.S. Doirea, S. Navickiene, Talanta, 76 (2008) 680.

[16] L. Lagunas-Allue, J. Sanz-Asensio, M.T. Martinez-Soria, Anal. Bioanal. Chem., 398 (2010) 1509.

[17] S. Wang, Y. Xu, C. Pan, S. Jiang, F. Liu, Anal. Bioanal. Chem., 387 (2007) 673.

[18] Association of Official Analytical Chemists, Official Methods of Analysis of the Association of Official Analytical Chemists, 1, 15th ed., Association of Analytical Chemists, 1990.

[19] L. Lagunas-Allue, J. Sanz-Asensio, M.T. Martinez-Soria, J. Chromatogr. A., 1270 (2012) 62.

[20] P. Labarta, M.P. Martínez-Moral, M.T. Tena, Anal. Chem., 2012 (2012) 8.

[21] E. Schreck, F. Geret, L. Gontier, M. Treilhou, Talanta, 77 (2008) 298.

[22] J.L.M. Vidal, J.A.P. Sanchez, P. Plaza-Bolaos, A.G. Frenich, R. Romero-González, J. AOAC Int. 93 (2010) 1715.

[23] T. Tanaka, T. Hori, T. Asada, K. Oikawa, K. Kawata, J. Chromatogr. A., 1175 (2007) 181.

[24] S.K. Cho, A.M. Abd El-Aty, J.H. Choi, Y.M. Jeong, H.C. Shin, B.J. Chang, C. Lee, J.H. Shim, J. Sep. Sci., 31 (2008) 1750.

[25] S.J. Lehotay, O.N. Collaborators: Mary, T. Jose, G. Antonio Valverde, C. Mariano, M. Hans, H. Volkmar, A. Thomas, L. Gnter, F. Richard, M. Katerina, P. Mette Erecius, B. Amy, H. Walter, C. Jo Marie, A. Lutz, L. Karen, G. Miguel, H. Marvin, K. Andr de, H. Maurice, S. Frank, W. Anthony, P. Alesia, J. AOAC Int., 90 (2007) 485.

[26] S. Walorczyk, aw, Dro, D. ski, J. AOAC Int., 94 (2011) 1625.

[27] A.N. Brown, J.M. Cook, W.T. Hammack, J.S. Stepp, J.V. Pelt, G. Gerard, J. AOAC Int., 94 (2011) 931.

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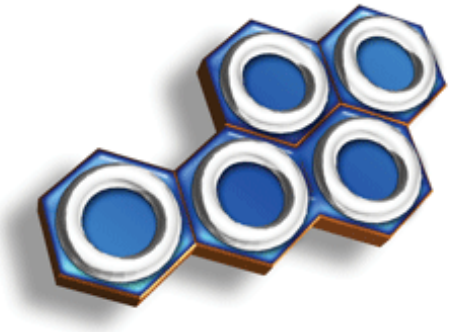
3. Determinación de hidrocarburos aromáticos policíclicos en superficies de juego y aceite de oliva



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Como ya se ha comentado en la introducción, los hidrocarburos aromáticos policíclicos (PAHs) son considerados por la EPA contaminantes prioritarios debido a sus efectos nocivos sobre la salud humana. Estos compuestos son contaminantes ubicuos, especialmente en atmósferas urbanas y se han encontrado en los neumáticos reciclados a partir de los que se fabrican las superficies empleadas en parques, campos de fútbol y zonas de juego infantiles.

El principal ***objetivo*** del ***Capítulo III*** de esta Tesis Doctoral es ***determinar la presencia de PAHs y otras sustancias potencialmente tóxicas*** (plastificantes, antioxidantes, antiozonantes), ***en*** estas ***superficies de caucho recicladas en ambientes interiores***, ***así como demostrar su transferencia tanto al aire como al agua*** que está en contacto con las superficies.

Para el análisis directo de las superficies de caucho recicladas se ha empleado la extracción asistida por ultrasonidos (*ultrasound assisted extraction, UAE*), mientras que para el estudio del aire y agua en contacto las mismas se ha empleado la SPME, en base a estudios previos del grupo de investigación en el que se ha desarrollado este trabajo, demostrando ser una técnica eficaz y robusta.

Por último, en este Capítulo se exponen esquemáticamente los resultados experimentales obtenidos durante la breve estancia realizada en la Universidad Técnica de Creta dentro del Laboratorio de Química Acuática dirigido por la Doctora Elefteria Psillakis. En este caso, siguiendo con las líneas de investigación del Laboratorio, se han optimizado las condiciones experimentales para el análisis de PAHs en aceite de oliva empleando Vac-HS-SPME-GC-MS. Esta técnica de extracción ha sido ampliamente desarrollada y aplicada por Psillakis y colaboradores al estudio de PAHs en suelos y aguas, pero nunca se había aplicado a otras matrices líquidas como aceites.

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3.1. INVESTIGATION OF PAH AND OTHER HAZARDOUS CONTAMINANT OCCURRENCE IN RECYCLED TYRE RUBBER SURFACES. CASE-STUDY: RESTAURANT PLAYGROUND IN AN INDOOR SHOPPING CENTRE

International Journal of Environmental Analytical Chemistry (Ed. Taylor & Francis Online, United Kingdom)
ISSN: 0306-7319

***Int. J. Environ. Anal. Chem., 12 (2014) 1464-1271*. doi: 10.1080/03067319.2014.930847**

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INVESTIGATION OF PAH AND OTHER HAZARDOUS CONTAMINANT OCCURRENCE IN RECYCLED TYRE RUBBER SURFACES. CASE-STUDY: RESTAURANT PLAYGROUND IN AN INDOOR SHOPPING CENTRE

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ABSTRACT

The objective of this case study was to investigate the presence of polycyclic aromatic hydrocarbons (PAHs) and other hazardous organic chemicals in a recycled tyre playground surface (in an indoor restaurant of a shopping centre with limited ventilation). This study also aimed at underlining both the volatilisation of these compounds in the vapour phase above the sample and the partial leaching of contaminants from the playground surface to the runoff and cleaning water put in contact with the sample. Playground samples were extracted with ethyl acetate using ultrasonic energy followed by GC-MS analysis. In addition, the same samples were analysed by HS-SPME to study the volatilisation and the transfer of those organic compounds. The analysis confirmed the presence of a large number of hazardous substances. Thus, 14 of the 16 studied PAHs were identified in the extracts (including the considered most toxic PAH, benzo[a]pyrene) and nine of them were also detected in the vapour phase. Besides, nine PAHs were found in the runoff/cleaning water, yielding a total PAH concentration at the ppm level. The presence and the high concentrations of these chemical compounds in playgrounds should be a matter of concern owing to their high toxicity.

Keywords: GC-MS; phthalates; playgrounds; polycyclic aromatic hydrocarbons (PAHs); recycled tyre rubber surfaces; runoff water; SPME.

1. INTRODUCTION

Today, one of the most valuable applications of used tyres is the transformation in recycling products such as recycled rubber pavers that are used for asphalt pavements, animal flooring, and parking or playground surfaces among others. It is well known that rubber tyres contains toxic compounds such as antioxidants, plasticisers, antiozonants or softeners among other chemicals and, in last years, several studies have warned of the toxicity of tyre rubber recycled products [1,2].

The presence of hazardous organic chemicals, including high polycyclic aromatic hydrocarbons (PAH) levels, in recycled tyre playground surfaces have been recently demonstrated [3,4]. The United States Environmental Protection Agency (US EPA) has classified 16 of these compounds as priority-pollutants based on toxicity, potential for human exposure and frequency of occurrence at hazardous wastes [5]. Of these PAHs, the US EPA considers seven of them (benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, benzo[k]fluoranthene and dibenz[a,h]anthracene) as probable human carcinogens [6].

PAHs occur naturally in coal and petroleum products and are formed by the incomplete combustion of organic matter. They are ubiquitous contaminants in urban environments, where they have sources such as automobile or industrial atmospheric emissions, asphalt or tyre particles [7,8]. The latter are employed as rubber mulch in playground flooring. This product consists of granular rubber particles and constitutes a slip-resistant floor that prevents injuries in sporting activities and playgrounds, being at the same time a visually attractive choice.

These kinds of surfaces are mainly used outdoor, where rainwater can accumulate and wash the surfaces. Water runoff can transport PAHs and other organic compounds and then transfer them to environment compartments (e.g. surface water, soils) [9-11]. In addition, in indoor environment, these surfaces are frequently ‘cleaned’ with aqueous mixtures (e.g. small amounts of detergent diluted in water), which enter the wastewater cycle.

The objective of this study was to investigate the presence of 16 EPA priority PAHs and other hazardous organic chemicals in a recycled tyre playground surface. This study also aimed at demonstrating the partial transfer of contaminants from the rubber surface to the surrounding air and to water put in contact with the sample. As a case study, an indoor playground sample from a restaurant in a shopping centre was selected. Actually, the potential risk for the infants is likely to be higher in these confined atmospheres devoted to “eat and play”. The analysed samples consisted of two different types of ground covers of two colours: the coloured upper layer (green) and a bottom layer (black). As regards analytical methodology, samples were extracted with ethyl acetate using ultrasonic energy followed by gas chromatography-mass spectrometry (GC-MS) analysis. In addition, the playground sample was analysed by head-space solid-phase microextraction (HS-SPME) exposing the fibre (either polydimethylsiloxane (PDMS) or divinylbenzene (DVB)) to the headspace over the sample for 30 min. SPME studies of the vapour phase above the samples and of runoff water allow assessing the volatilisation and the transfer of those organic compounds.

2. EXPERIMENTAL

2.1 Reagents and material

The studied compounds, their chemical names and CAS numbers are summarised in **Table 1**. Ethyl acetate was provided by Sigma-Aldrich (Steinheim, Germany). The SPME manual holders and 65 µm PDMS or DVB were supplied by Supelco short (Bellefonte, PA, USA). Individual stock solutions of each compound were prepared in acetone. Further dilutions and mixtures were prepared in ethyl acetate and then stored in amber glass vials at -20°C.

Table 1. Target compounds, chemical names, CAS number, suppliers, retention time and selected MS ions.

Key	Compound	CAS number	Retention time (min)	**Quantification** and identification ions[a]
PAHs[b]				
NAP	Naphtalene	91-20-3	5.88	127 (13), **128** (100), 129 (11)
ACY	Acenaphtlylene	208-96-8	7.23	150 (14), 151 (20), **152** (100)
ACE	Acenaphthene	83-32-9	7.40	152 (47), **153** (100), 154 (95)
FLU	Fluorene	86-73-7	7.91	165 (91), **166** (100), 167 (14)
PHN	Phenanthrene	85-01-8	9.03	176 (18), **178** (100), 179 (15)
ANC	Anthracene	120-12-7	9.10	176 (18), **178** (100), 179 (15)
FLA	Fluoranthene	206-44-0	11.35	200 (20), **202** (100), 203 (17)
PYR	Pyrene	129-00-0	11.87	200 (20), **202** (100), 203 (18)
B[a]A	Benz[a]anthracene	56-55-3	15.49	226 (26), **228** (100), 229 (19)
CHY	Chrysene	218-01-9	15.62	226 (28), **228** (100), 229 (20)
B[b]F	Benzo[b]fluoranthene	205-99-2	19.39	250 (22), **252** (100), 253 (22)
B[k]F	Benzo[k]fluoranthene	207-08-9	20.34	250 (22), **252** (100), 253 (22)
B[a]P	Benzo[a]pyrene	50-32-8	20.51	250 (23), **252** (100), 253 (22)
IND	Indeno[1,2,3-cd]pyrene	193-39-5	24.48	274 (20), **276** (100), 277 (24)
D[ah]A	Dibenz[a,h]anthracene	53-70-3	24.68	276 (26), **278** (100), 279 (24)
B[ghi]P	Benzo[ghi]perylene	191-24-2	25.46	274 (21), **276** (100), 277 (24)
Other compounds				
BTZ	Benzothiazole[c]	95-16-9	6.08	69 (15), 108 (30), **135** (100)
TBP	4-tert-butylphenol[c]	98-54-4	6.35	107 (36), **135** (100), 150 (21)
BHA	Butylated hydroxyanisole[c]	121-00-6	10.13	137 (64), **165** (100), 180 (51)
BHT	Butylated hydroxytoluene[c]	128-37-0	10.20	177 (7), **205** (100), 220 (25)
DMP	Dimethyl phthalate[d]	131-11-3	7.13	77 (16), **163** (100), 164 (10)
DEP	Diethyl phthalate[d]	84-66-2	7.78	105 (8), **149** (100), 177 (23)
DIBP	Diisobutyl phthalatc[e]	84-69-5	9.21	104 (7), **149** (100), 223 (7)
DBP	Dibutyl phthalate[c]	84-74-2	10.08	104 (4), **149** (100), 223 (5)
BBP	Benzylbutyl phthalate[c]	85-68-7	14.07	91 (55), **149** (100), 206 (24)
DEHA	Di(2-ethylhexyl)adipate[e]	103-23-1	14.48	112 (25), **129** (100), 147 (19)
DEHP	Di(2-ethylhexyl)phthalate[d]	117-81-7	16.40	**149** (100), 167 (32), 279 (11)
DOP	Di-n-octyl phthalate[d]	117-84-0	18.78	**149** (100), 223 (22), 279 (8)
DINP	Diisononyl phthalate[c]	28553-12-0	19.59	**149** (100), 167 (7), 293 (17)
DIDP	Diisodecyl phthalate[c]	26761-40-0	20.81	**149** (100), 167 (10), 307 (20)

[a] Numbers in brackets are the relative ion abundances, %. [b] 16 PAHs mixture (2000 µg mL^{-1} in dichloromethane/benzene, 1:1) purchased from Ultra Scientific Analytical Solutions (Kingstown, USA). [c] Sigma-Aldrich Chemie GmbH (Steinheim, Germany). [d] Fluka Chemie GmbH (Steinheim, Germany). [e] ChemService (West Chester, Germany).

2.2 Sampling and sample treatment

An indoor playground from a restaurant in a shopping centre was selected. The samples consisted of two different types of ground covers of two colours: the coloured upper layer highly compacted (green) and a bottom layer with a lower compaction degree (black) which was also analysed. The samples were cut into small particles (around 0.3 cm of diameter) and stored in clean glass vials.

2.3 Ultrasound-assisted extraction (UAE)

Two millilitres of ethyl acetate were added to a 10 mL glass vial containing 200 mg of sample and sealed with a headspace aluminium cap furnished with PTFE-faced septum. The analytes were extracted from the samples to the organic solvent using ultrasonic energy (J.P. Selecta ultrasound bath, Barcelona, Spain) at 50 kHz frequency and 110 W of power. The mixture playground sample-solvent was sonicated (20 min) at 25°C. Afterwards, the supernatant was filtered through 0.45-µm PTFE microporous filters (25 mm diameter). The extract, diluted 1:10 (v/v) in ethyl acetate, was analyzed by GC-MS.

2.4 Solid-phase microextraction (SPME)

For the analysis of the vapour phase above the samples, 0.2 g of sample was transferred to a 10 mL glass vial. The vial was sealed and immersed in a thermostatised water bath (25°C or 60°C). The sample was let to equilibrate for 5 min before the exposition of a PDMS and/or PDMS/DVB fibre took place in the headspace over the sample (30 min).

For the analysis of the runoff water, 5 mL of Milli-Q water was added to a 10 mL glass vial containing 0.5 g of playground sample that was immersed in water for at least 2 hours. Afterwards, the supernatant (4 mL) was filtered and transferred into another vial, which was immersed in a water bath (100°C). The PDMS/DVB fibre was exposed to the headspace (30 min).

In both cases, once finished the preselected exposure time, the fibre was retracted into the needle of the holder syringe and immediately inserted into the GC injector. Desorption was carried out at 270°C under the selected conditions.

2.5 GC-MS analysis

The GC-MS analysis was performed using an Agilent 7890A coupled to an Agilent 5975C inert mass spectra detector (MDS) with triple axis detector and an Agilent 7693 autosampler from Agilent Technologies (Palo Alto, CA, USA). The temperatures of the transfer line, the quadrupole and the ion source were set at 290, 150 and 230°C, respectively. The system was operated by Agilent MSD ChemStation E.02.00.493 software. Separation was carried out on a TR-5 MS (30m×0.25mm i.d., 0.25 µm film thickness) obtained from Thermo Scientific (Palo Alto, CA, USA). Helium (purity 99.999%) was employed as carrier gas at 1.0 mL min^{-1}. The GC oven temperature was programmed from 60°C

(held 2 min) to 210°C at 15°C min^{-1} and a final ramp to 290°C (held 17 min) at 5°C min^{-1}. Splitless mode was used for injection and the injector temperature was kept at 270°C. The injection volume was 1 µL.

3. RESULTS AND DISCUSSION

GC-MS conditions for the analysis of PAHs and other target compounds are summarised in **Table 1**. This method has been previously validated by Llompart et al. [3] for the determination of hazardous organic chemicals in rubber recycled tyre playgrounds and pavers.

3.1 Analysis of recycled tire rubber surface (UAE)

Individual and total PAH contents in the playground sample are summarized in **Table 2.** Fourteen out of the sixteen EPA target PAHs were identified in the extracts (**Figure 1**).
In the external face of the playground surface (green colour), phenanthrene (PHN), pyrene, (PYR) and fluoranthene (FLA) were the most abundant PAHs with concentrations of 42, 34 and 25 µg g^{-1} respectively. Other targets PAHs were found at concentrations between 0.4-18 µg g^{-1}. Also, the considered most toxic PAH, benzo[a]pyrene (B[a]P), was found at quantifiable levels (6.4 µg g^{-1}). In this layer, the total target PAH concentration was 170 µg g^{-1} and three of the target analytes, indeno[1,2,3-cd]pyrene (IND), dibenz[a,h]anthracene (D[ah]A) and benzo [ghi]perylene (B[ghi]P) were not detected.

In the internal face of the playground surface (black colour), the total PAH concentration was 295 µg g^{-1}. Chrysene (CHY) was the most abundant PAH (62 µg g^{-1}). Phenanthrene (PHN), pyrene (PYR) and fluoranthene (FLA) were also detected and at higher concentration levels than in the external surface (52, 51 and 33 µg g^{-1}, respectively). Benzo[a]pyrene (B[a]P) was found at higher concentration than in the green cover (17 µg g^{-1}). Other PAHs were found at concentration levels ranging from 0.5 to 30 µg g^{-1}. In this case, benzo[k]fluoranthene (B(k)F), dibenz[a,h]anthracene (D[ah]A) and benzo [ghi]perylene (B[ghi]P) were not detected in the extracts.

These analyses also confirmed the presence of a high number of harmful compounds (**Table 2**) including plasticisers (phthalates and adipates), antioxidants, benzothiazole and derivatives, among other chemicals.

Regarding plasticisers, phthalates such as di(2-ethylhexyl)phthalate (DEHP) were detected at high levels in both types of sample (3045 and 4563 µg g^{-1} in the green and black surfaces respectively). In addition, diisobutyl phthalate (DIBP) was found at concentrations above 1000 µg g^{-1} in the green upper ground cover and above 3300 µg g^{-1} in the black bottom ground cover. Dibutyl phthalate (DBP) was also found reaching values above 600 µg g^{-1} on both surfaces. These compounds are forbidden in several applications such as cosmetics and personal care products, textile, childcare articles and toys. Other phthalates (diethyl phthalate, benzylbutyl phthalate and di n-octyl phthalate) were detected at concentrations below 52 µg g^{-1}. Di (2-ethylhexyladipate) was detected at

concentrations close to 100 µg g^{-1}. Other detected compounds were benzotiazole (BTZ) and butylated hydroxytoluene (BHT) at concentrations below 26 µg g^{-1}.

Table 2. Concentration (µg g^{-1}) of PAHs and other target compounds in the surface rubber samples.

PAHs	**Green cover**	**Black cover**
NAP	0.43	0.52
ACY	1.6	0.79
ACE	2.0	2.5
FLU	8.5	11
PHN	42	52
ANC	8.5	11
FLA	25	33
PYR	34	51
B[a]A	12	30
CHY	18	62
B[b]F	10	18
B[k]F	2.2	n.d
B[a]P	6.4	17
IND	n.d	5.8
Total PAHs	***170***	***295***
Other compounds	**Green cover**	**Black cover**
BTZ	26	24
BHT	10	11
DEP	0.62	0.65
DIBP	1463	3314
DBP	604	1319
BBP	41	19
DEHP	3045	4563
DNOP	14	52
DEHA	94	140

n.d: not detected

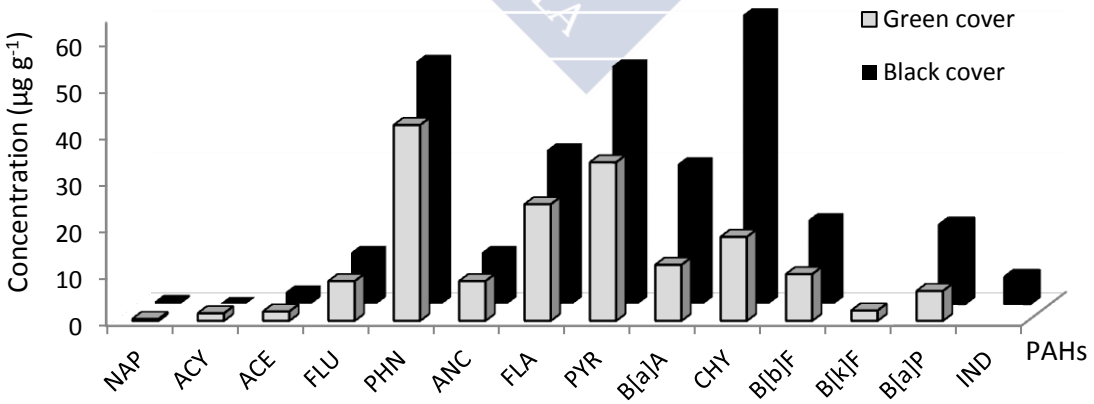

Figure 1. **Comparison between PAH concentration (µg g^{-1}) in green and black playground sample surface**

3.2 Headspace-solid phase microextraction (HS-SPME) studies

The playground samples were also analysed by HS-SPME. Two extraction temperatures, 25 and 60°C were tested, obtaining higher response at 60°C (**Figure 2**). Nine of the fourteen PAHs found in the playground sample were identified in the vapour phase, excluding the less volatile ones. All the compounds detected in the experiments at 60ºC were also found at ambient temperature (25°C). These experiments demonstrated that these chemicals can reach the vapour phase and thereby enter the human organism by inhalation.

Many other analytes such as BTZ, BHT, DIBP and DBP were identified in the headspace. Other non-target compounds such as acetophenone, Flectol H, Tri(2-ethylhexyl)trimellitate, Bis(2-ethylhexyl)sebacate, Dibenzofurane, Triallylisocyanurate, Benzene,1,3-diisocyanate-2-methyl (2,6TDI), and 2,5.di-tert-butylphenol, were tentatively identify on the basis of the similarity (mass fragments and abundance) between the experimental mass spectra and the Wiley library mass spectra.

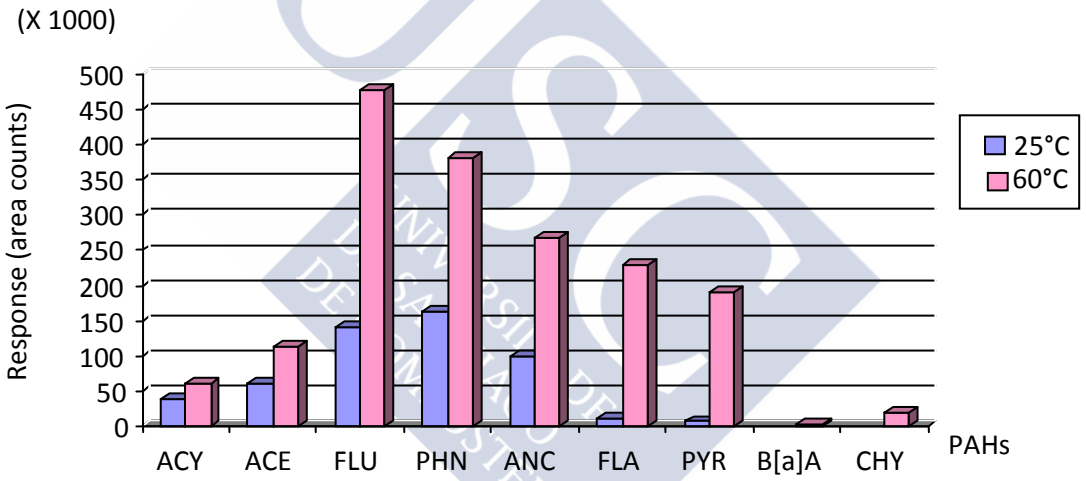

Figure 2. PAHs identified in the vapour phase. Comparison between responses at 25°C and 60°C by HS-SPME.

Additional studies proved the partial leaching of some target compounds from the playground tyre surface to the water put in contact with the sample. By this method, nine of the sixteen PAHs studied were found in water after leaching (**Table 3**). Phenanthrene (PHN) and benz[a]anthracene (B[a]A) were the most abundant PAH at levels of 709 and 681 ng mL^{-1}, respectively. Other seven PAHs were identified with concentrations between 5.2 ng mL^{-1} (naphthalene, NAP) and 243 ng mL^{-1} (fluoranthene, FLA). The total PAH concentration reached in water was at the ppm level. Other target organic compounds such as benzothiazole (BTZ) or phthalates (DBP, DIBP and DEHP) were also found in the water; BTZ was detected at levels about 18 ng mL^{-1}; DBP at concentration close to 64 ng mL^{-1} and DIBP and DEHP at higher levels (233 and 613 ng mL^{-1} respectively).

Table 3. Concentration (ng mL^{-1}) of PAHs and other compounds found in water afeter leaching

PAHs	**Concentration**
NAP	5.2
ACY	26
ACE	15
FLU	111
PHN	709
ANC	211
FLA	243
PYR	222
B[a]A	681
Total PAHs	***2223***
Other compounds	**Concentration**
BTZ	18
DIBP	233
DBP	64
DEHP	613

4. CONCLUSIONS

In this case-study, fourteen out of the sixteen EPA priority PAHs were identified and quantified in the investigated recycled tyre rubber playground surfaces. The analytical measurements also confirmed the presence of other harmful compounds including phthalates, adipates, antioxidants and benzothiazole among others, in some cases at high concentration levels (DEHP> 3000 μg g^{-1}).

HS-SPME studies demonstrated the presence of nine PAHs in the vapour phase above the playground sample, showing that these chemicals could reach the surrounding air. Consequently, PAHs would likely be accessible by inhalation increasing the risk to children.

As these kinds of surfaces are also used outdoor, rainwater can accumulate and wash the surfaces before being discharged to different environmental compartments. Besides, this study proved that a contaminant leaching occurred from the playground to the water put in contact with the samples. In this way, nine of the sixteen target PAHs were detected in water, yielding a total PAH concentration at the ppm level.

Acknowledgements

This research was supported by FEDER funds and project CN 2012/299 (Consolidated Research Groups Program, Xunta de Galicia).

References

[1] D.A. Birkholz, K.L. Belton, T.L Guidotti, J. Air waste Manage. Associat. 53 (2003), 903.

[2] J.M. Rooij, F. Jongeneelen, Int. Arch. Occup. Environ. Health 83 (2010) 105.

[3] M. Llompart, L. Sanchez-Prado, J.P. Lamas, C. Garcia-Jares, E. Roca, T. Dagnac, Chemosphere 90 (2013), 423.

[4] R.T Ottesen, J. Alexander, M. Langedal, T. Haugland, E. Høygaard, Environ. Geochem. Health 30 (2008), 623.

[5] http://www.water.epa.gov/scitech/methods/cwa/pollutants.cfm, accesed on March 2014

[6] http://www.epa.gov/nata2002/gloss.htlm, accessed on March 204

[7] G. Purcaro, S. Moret, L.S. Conte, Talanta 105 (2013) 292.

[8] X. Wang, S. Liu, J. Zhao, Q. Zuo, W. Liu, B. Li, S. Tao, Environ. Toxicol. Chem. 33 (2014) 753.

[9] B. J. Mahler, P.C.V. Metre, J.L Crane, A.W. Watts, M. Scoggins, E.S. Williams, Environ. Sci. Technol. 46 (2012) 3039.

[10] J. Crane, Arch. Environ. Contam. Toxicol. 66 2014 176.

[11] E. Garcia-Flores, J.H. Espinoza-Gomez, Int. J. Environ. Res. 7 (2013) 387.

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3.2. OPTIMIZATION OF THE EXPERIMENTAL CONDITIONS FOR THE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN OLIVE OIL BY VACUUM-SOLID-PHASE MICROEXTRACTION

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OPTIMIZATION OF THE EXPERIMENTAL CONDITIONS FOR THE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN OLIVE OIL BY VACUUM-SOLID-PHASE MICROEXTRACTION

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1. INTRODUCTION

This work was developed during the short stay at the Technical University of Crete. Its aim was the selection of the optimal experimental conditions for a vacuum-solid-phase microextraction (Vac-SPME) method to determine the most volatile PAHs in olive oil. In this way, preliminary experiments were carried out and different conditions were tested in order to obtain the highest extraction efficacy.

Olive oil is an important component of the Mediterranean diet and its consumption is believed to be beneficial to human health. The high solubility of polycyclic aromatic hydrocarbons (PAHs) in organic matrices leads their accumulation in edible oils and fats, which may be contaminated by environmental pollution or during processing steps prior to refining. Several studies have reported the presence of PAHs in olive oil [1-3]. European legislation establishes a maximum permitted concentration of 2 μg kg^{-1} for benzo(a)pyrene, and 10 μg kg^{-1} to the sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, and chrysene. Although Spain, Greece and Italy reduce this value to 5 μg kg^{-1}.

2. EXPERIMENTAL

2.1. Selected polycyclic aromatic hydrocarbons

Although reduced pressure conditions during HS-SPME sampling are not expected to increase the amount of analytes extracted at equilibrium, they may drastically improve extraction kinetics compared to regular HS-SPME during the non-equilibrium stage of the sampling process due to the enhancement of evaporation rates in the presence of air-evacuated headspace. Acceleration effects on extraction rates induced by reducing the total pressure of the sample container are expected to be important when the Henry constant (K_H) value is close to or below the reported threshold values for low K_H solutes ($1.2x10^{-5}$ or $1.6x10^{-4}$ atm m^3 mol^{-1}) [4-6]. For these compounds, mass transfer resistance in the thin gas-film adjacent to the gas/sample interface controls more than 95% of the evaporation rates and hence, reducing the total pressure will result in a faster overwall extraction process [4-6]. On the other hand, for intermediate K_H compounds (K_H between the above mentioned threshold values and less than $5x10^{-3}$ atm m^3 mol^{-1}, Vac-HS-SPME is not expected to improve extraction rates compared to conventional HS-SPME since mass transfer resistance located

in the thin liquid-film controls evaporation rates and this process is independent of the pressure conditions in the headspace [7-9].

According to the K_H values presented in **Table 1**, napthalene (NAP) represents the case of an intermediate K_H compound. Acenaphthene (ACE), fluorene (FL), and phenanthrene (PHN) lie on the border between intermediate and low K_H compounds, and fluoranthene (FLU) represents the low K_H class of compounds.

Table 1. CAS number, some physicochemical properties and quantification ions of the five studied PAHs

Compounds	Abreviation	CAS number	Molecular weight	K_H (atm m^3 mol^{-1})	Quantification ions
Naphthalene	NAP	91-20-3	128.2	4.4×10^{-4}	128
Acenaphthene	ACE	83-32-9	152.2	1.8×10^{-4}	153
Fluorene	FL	86-73-7	166.2	9.6×10^{-5}	166
Phenanthrene	PHEN	85-01-8	172.2	4.2×10^{-5}	178
Fluoranthene	FLU	206-44-0	202.3	8.9×10^{-6}	202

2.2. Vac-SPME procedure

A 22 mL glass vial sealed with a Teflon cap with a Thermogreen septum (Supelco, Bellefonte, PA, USA), and containing a cylindrical Teflon-coated magnetic stir bar (9 mm x 3 mm) was air-evacuated connecting trough a syringe a vacuum pump (Vacuubrand GmbH & Co.KG, model MZ 2C NT, Wertheim, Germany). After the air-evacuation, 2 g of olive oil spiked at 20 ng g^{-1} with target compounds, were introduced into the vial through the Thermogreen septum with a 10 mL gastight syringe (SGE; Australia). Then, the vial was immersed in a water bath (different temperatures were tested in order to optimize the extraction) and was maintained under magnetic stirring (1400 rpm) during 10 minutes to equilibrate. Afterwards, the fiber (PDMS or DVB/CAR/PDMS) was exposed to the head space (various exposition times were also tested in order to improve the responses). In all cases, once finished the selected exposition time, the fiber was retracted into the needle of the holder syringe and inserted into the GC injector. Desorption was carried out at 260°C under the selected conditions.

2.3. GC-MS analysis

The GC-MS analysis was performed using a Varian 450-GC coupled to a Varian 240-MS (Varian, Walnut Creek, CA, USA). The temperatures of the manifold, ion trap, ion source and transfer line were set at 50, 150, 180, an 260°C, respectively. The system was operated by Saturn GC-MS Workstation v6.9 software. Separation was carried out on a VF 5 MS capillary column (30m×0.25mm i.d., 0.25 µm film thickness) obtained from Bruker (Netherlands). The GC oven temperature was programmed from 75°C (held 2 min) to 150°C at 25°C min^{-1} (held 2 min) and a final ramp to 300°C (held 5 min) at 15°C min^{-1} (total analysis time, 22 min). A 5 minutes solvent delay time was used. Helium (purity 99.999%) was employed as carrier gas at 1.0 mL min^{-1}. The ion trap mass spectrometer was operated in the electron impact (EI) ionization positive mode (+70 eV) using an external ionization configuration. The mass spectra detector operated in selected ion storage (SIS) mode. Acquisition of data was divided in five ion sets, monitoring two or three ions per compound in each one.

2.4. Optimization of the experimental conditions

Several experimental conditions were tested in order to obtain the higher extraction efficiency for Vac-HS-SPME. The optimizations were carried out with 2 grams of olive oil spiked at 20 ng g^{-1} with each target analyte. The studied parameters and levels are summarized in **Table2**.

Table 2. Tested parameters and studied levels for the Vac-HS-SPME procedure

Tested parameters	Studied levels
SPME fibers	PDMS and DVB/CAR/PDMS
Vacuum time	(15, 30, 40, 60) seconds
Equilibrium time (before extraction)	(5, 10, 15) minutes
Extraction time	(5, 10, 15, 20, 30, 40, 50, 60) minutes
Extraction temperature	(25, 40, 60, 80, 100)°C

Effect of the fiber and extraction temperature

Two types of fibers, 100-µm PDMS, and the triple 50/30-µm DVB/CAR/PDMS were tested at different temperatures exposing the fiber to the head space 30 minutes, after 10 minutes of equilibrium time.

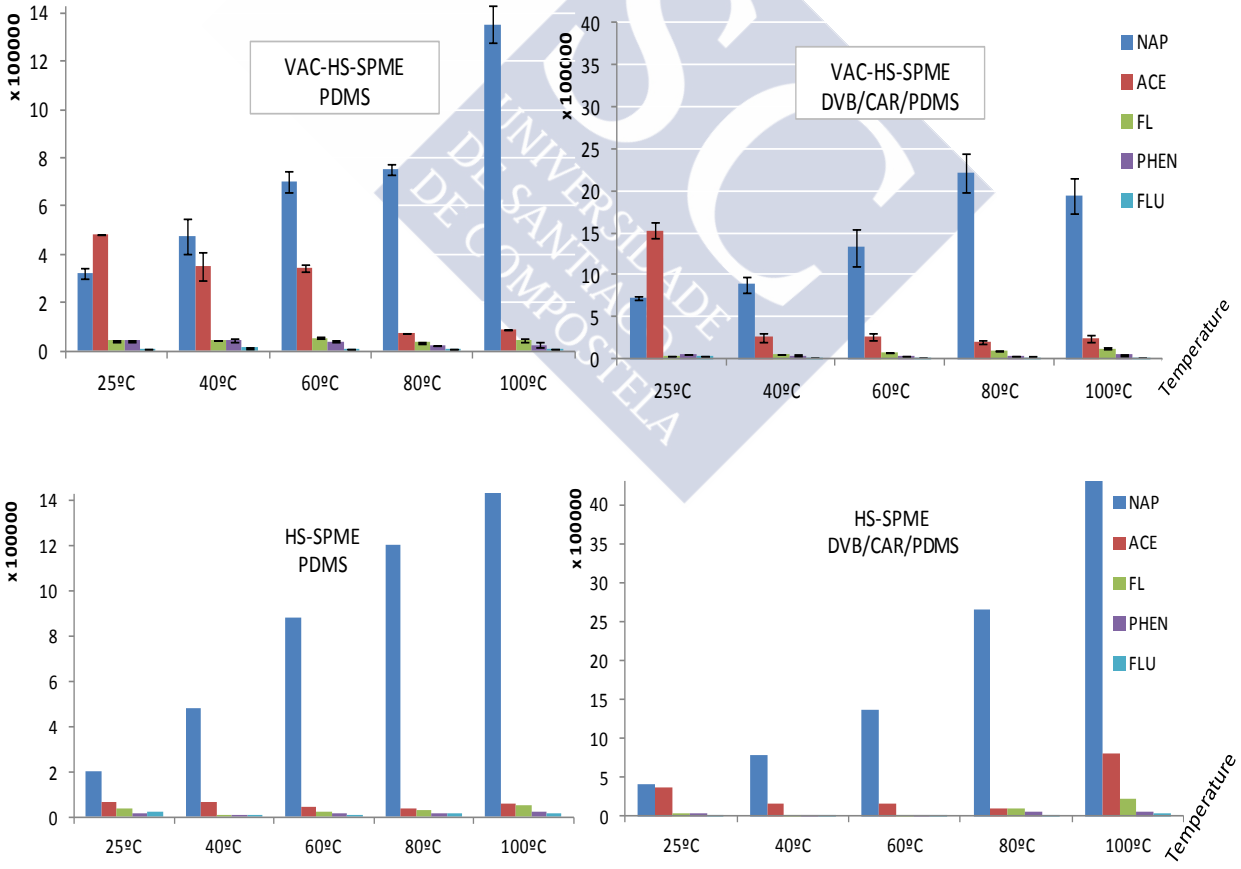

Figure 1. Comparison between Vac-HS-SPME and HS-SPME for two fibers at different temperatures

Combining the effects of temperature and reduced pressure in Vac-HS-SPME, was expected to enhance even further the kinetics of the extraction up to certain temperature above which the effect of temperature would dominate the extraction. As shows **Figure 1**, from 60°C an exponential decrease for all compounds is observed, excluding the most volatile (NAP) for Vac-HS-SPME. Based on these results it was decided to use 25°C as sampling temperature, and PDMS was selected as SPME fiber due to its high efficiency to extract target compounds at the selected temperature.

Effect of the equilibrium time

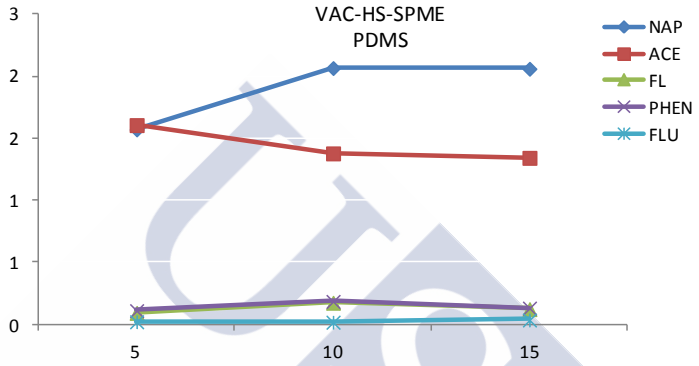

Figure 2. Effect of the equilibrium time for the studied PAHs

All experiments were carried out applying agitation, since equilibrium times are shortened working under constantly stirring. No significant differences were obtained at 5, 10 or 15 minutes as equilibrium time before the fiber exposition, so finally, the intermediate level (10 minutes) was selected as equilibrium time.

Effect of extraction time

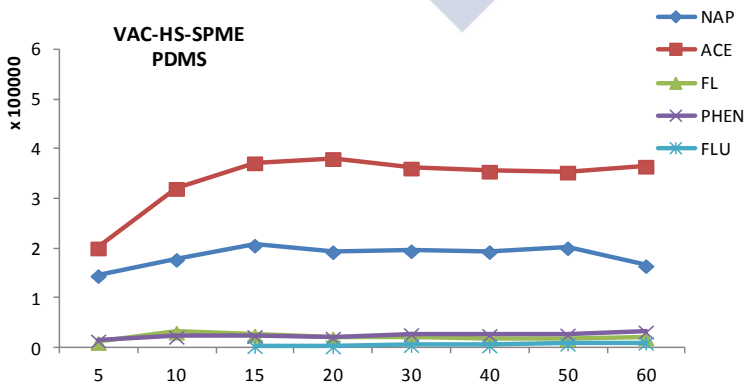

Figure 3. Effect of the extraction time for the studied PAHs

Figure 3 shows the extraction time profiles working with Vac-HS-SPME. For the most volatile compounds (NAP, ACE, FL) equilibrium is obtained after 15 minutes of extraction. On the contrary, PHEN and FLU were far from equilibrium even after sampling for 60 minutes. In view of the results, 20 minutes were selected as extraction time, since SPME is completed when the analyte reaches distribution equilibrium between the sample and the fiber coating, and longer extraction time will not result in further increase in the analytes amount extracted.

Effect of the air evacuation time

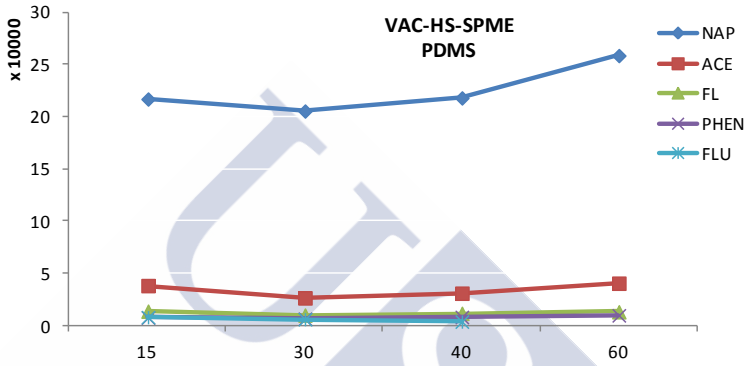

Figure 4. Effect of the air evacuation time

Evacuating the air from the vial before rather than after sample introduction esures repeatability of the process and eliminates the possibility of analyte losses due to the air evacuation of the headspace in the presence of the sample. As shows **Figure 4** no significant differences were observed between 15, 30, 40 and 60 seconds, so finally 30 seconds were selected as air evacuation time.

In view of these results, the selected conditions to analyze the most volatile PAHs in olive oil are summarized in **Table 3.** They were PDMS fiber, at 25°C with 10 minutes of equilibrium, and exposing the fiber to the head space during 20 minutes, with a previous vacuum time of 30 seconds.

Table 3. Selected conditions for the Vac-HS-SPME analysis

Tested parameters	Selected conditions
SPME fibers	PDMS
Vacuum time	30 seconds
Equilibrium time (before extraction)	10 minutes
Extraction time	20 mintes
Extraction temperature	25°C

References

[1] S. Moret, S.C. Lanfranco, J. Chromatogr. A., 882 (2000) 245.

[2] V.H. Teixeira, S. Casal, M.B. Oliveira, Food Chem., 104 (2007) 106.

[3] D. Costopoulos, I. Vassiliadou, D. Chrysafidis, B. Bergele, E. Tzavara, V. Tzamtis, L. Leondiadis, Chemosphere 79 (2010) 285.

[4] J.H. Smith, D.C Bomberg, D.L. Haynes, Chemosphere, 10 (1981) 281.

[5] J.H. Smith, D.C Bomberg, D.L. Haynes, Environ. Sci. Technol. 14 (1980) 1332.

[6] D. Mackay, P.J. Leinonen, Environ. Sci. Technol. 9 (1975) 1178.

[7] E. Psillakis, A. Mousouraki, E. Yiantzi, N. Kalogerakis, J. Chromatogr. A., 1244 (2012) 55.

[8] E. Psillakis, E. Yiantzi, L. Sanchez-Prado, N. Kalogerakis, Anal. Chim. Acta., 742 (2012) 30.

[9] E. Psillakis, E. Yiantzi, N. Kalogerakis, J. Chromatogr. A., 1300 (2013) 119.

IV. CONCLUSIONES - CONCLUSIONS

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IV. CONCLUSIONES-CONCLUSIONS

Tras la presentación y discusión de los resultados obtenidos en los trabajos que engloban esta Tesis Doctoral, a continuación se presentan las conclusiones más relevantes obtenidas para cada uno de ellos.

CAPÍTULO I: DETERMINACIÓN DE SUSTANCIAS POTENCIALMENTE TÓXICAS EN PRODUCTOS DE CUIDADO PERSONAL

1.1. Análisis de plastificantes y almizcles sintéticos en cosméticos y productos de cuidado personal mediante dispersión de matriz en fase sólida seguida de cromatografía de gases-espectrometría de masas

Este estudio fue el primero que se ocupó del análisis simultáneo de un elevado número de plastificantes (ftalatos y adipatos) y musks en productos cosméticos tanto de aclarado como de permanencia sobre la piel

Como técnica de extracción se empleó, también por primera vez para el análisis de estos compuestos, la dispersión de matriz en fase sólida (MSPD) que fue miniaturizada con el objetivo de minimizar el riesgo de contaminación por ftalatos, asi como para reducir residuos y costes. Esta microtécnica se realizó con material de uso común en laboratorio y apenas generó residuos, ya que las cantidades empleadas de muestra y disolventes orgánicos fueron 0,1g. y 1 mL, respectivamente.

Las condiciones de extracción se optimizaron mediante diseños experimentales sobre muestras reales, tanto de aclarado como de permanencia con el objetivo de obtener un método que pudiese ser aplicado a un amplio rango de muestras cosméticas. Se estudiaron el tipo de muestra (aclarado o permanencia), el tamaño de muestra (0,1 o 0,5 g.) y el disolvente de elución (hexano:acetona o acetato de etilo). Las condiciones finalmente seleccionadas fueron 0,1 g. de muestra y acetato de etilo como disolvente de elución para ambos tipos de muestras.

La validación se llevó a cabo en términos de linealidad (R> 0,9954), exactitud (recuperaciones entre 85-105%) y precisión (RSD <8%). Los LODs obtenidos se sitúan en niveles de la baja ppb, excluyendo los ftalatos DIHP, DINP y DIDP (50, 120 y 300 ng g^{-1}) que están formados por un elevado número de isómeros, por lo que su señal cromatográfica está formada por varios picos cromatográficos, lo que explica sus valores de LODs.

Por último, una vez validado, el método propuesto se aplicó a 26 muestras comerciales de muy diversa naturaleza. Veinticinco de los 30 compuestos estudiados se encontraron en las muestras, incluyendo concentraciones elevadas (hasta 141 µg g^{-1}) de algunos compuestos prohibidos como DBP o DEHP. Hay que destacar que ninguno de los compuestos estudiados aparece en la lista de ingredientes, ya que la legislación europea no lo requiere y su presencia se indica bajo el término “fragancia” o “perfume”.

1.2. Desarrollo de un método multianalito basado en micro-dispersión de matriz en fase sólida para el análisis de fragancias y conservantes en productos de cuidado personal.

En este caso se aplicó la µ-MSPD como técnica de extracción para el análisis simultáneo de 38 compuestos presentes habitualmente en productos cosméticos, como son las fragancias alergénicas y los conservantes. La mayoría de ellos se encuentran regulados por la Unión Europea en términos de máxima concentración permitida y algunos se encuentran prohibidos. Las condiciones de la etapa de extracción se optimizaron, tanto para muestras de aclarado como de permanencia, y las condiciones finalmente seleccionadas fueron 1mL de acetato de etilo como disolvente de elución y Florisil como dispersante. El tamaño de la muestra se fijó en 0,1 g en base al trabajo presentado anteriormente.

Para la determinación simultánea de los 38 compuestos estudiados se utilizó GC-MS y GC-MS/MS. Se empleó MS/MS ya que las matrices cosméticas son de naturaleza muy compleja y en muchos casos contienen ingredientes con estructuras similares a las de los analitos que se pretenden determinar (mismos iones), por lo que la selectividad y sensibilidad se pueden ver reducidas por estas "interferencias". Trabajando con MS/MS se reducen e incluso eliminan las interferencias de matriz, obteniéndose mayor selectividad y sensibilidad analítica. Hay que destacar que es la primera vez que se emplea GC-MS/MS para la determinación simultánea de fragancias alergénicas y conservantes en un amplio número de muestras cosméticas. Asímismo, para el análisis de los conservantes se incluyó una etapa de derivatización lo que permitió una mejora en la forma de pico cromatográfico, lo que implica una disminución en sus LODs.

Ambos métodos, GC-MS y GC-MS/MS han sido validados mostrando buena linealidad, sensibilidad, exactitud y precisión, con recuperaciones medias del 90% y RSD< 15%. Con el uso de MS/MS se obtuvieron LODs hasta 1 orden de magnitud menores que con MS. Para los conservantes, aunque todos fueron exitosamente analizados sin la etapa de derivatización, la inclusión de esta permitió mejorar (hasta 1 orden de magnitud) sus LODs.

Por último, el método fue aplicado al análisis de gran variedad de muestra cosméticas de muy diversa naturaleza. La mayoría de los compuestos analizados se encontraron presentes en las muestras, destacando la presencia de algunos conservantes en los límites legales que establece la normativa europea. Asímismo, un 25% de las muestras analizadas no cumplió con los requerimientos de etiquetado.

1.3. Dispersión de matriz en fase sólida en vial para el análisis de fragancias alergénicas, conservantes, plastificantes y musks en cosméticos.

En base a los trabajos anteriores, se planteó la idea de desarrollar un método multianalito basado en µ-MSPD-GC/MS para determinar simultáneamente por primera vez cerca de 70 compuestos que pueden formar parte de la formulación de productos cosméticos. Las condiciones de extracción fueron optimizadas en los dos trabajos anteriores e implican el uso de 0,1 g de muestra y 1 mL de acetato de etilo como disolvente de elución.

Al observar las bajas recuperaciones obtenidas para las fragancias alergénicas más volátiles (*pinene* y *limonene*), se planteó la idea de realizar la dispersión de la muestra en el propio vial donde

se pesa la muestra, para minimizar las pérdidas de estos analitos y, asimismo, reducir el número de pasos en las etapa de extracción. De esta forma, se comparó el procedimiento "clásico" en mortero y este nueva propuesta "en vial", obteniéndose de esta última forma, recuperaciones cuantitativas para todos los compuestos estudiados.

El método fue validado mostrando excelente linealidad, repetibilidad, reproducibilidad, exactitud y precisión. Por último fue aplicado a muestras cosméticas comerciales, donde se detectaron 48 de los 66 compuestos estudiados, algunos de ellos sobrepasando los límites legales.

Por lo que se puede concluir que la **µ-MSPD seguida de GC-MS** desarrollada en estos trabajos puede ser ***aplicada con éxito a muestras cosméticas de muy diversa naturaleza*** para ***determinar de forma rápida, a bajo coste y con mínima generación de residuos***, un ***gran número de compuestos de*** familias tan distintas como ***fragancias, conservantes y plastifcantes***. Además, el empleo de MS/MS permite obtener mayor selectividad, al reducir e incluso eliminar las posibles interferencias que a menudo ocurren al analizar muestras tan complejas como las cosméticas.

1.4. Extracción con líquidos presurizados-cromatografía de gases-espectrometría de masas para el análisis de fragancias alergénicas, musks, ftalatos y conservantes en toallitas infantiles.

Siguiendo con la temática de este capítulo del estudio de productos de cuidado personal, se ha desarrollado un método basado en la extracción con líquidos presurizados (PLE) seguido de GC/MS para el análisis de 65 sustancias potencialmente tóxicas en toallitas infantiles y papel higiénico húmedo destinado a niños menores de 3 años. La idea de este estudio surgió al comprobar que no existe ningún método de análisis para este producto tan específico y destinado a una población tan vulnerable como la infantil.

Debido a la naturaleza de las toallitas infantiles, se empleó PLE como técnica de extracción y el procedimiento fue optimizado mediante diseños experimentales para obtener la máxima eficacia. Las condiciones seleccionadas fueron MeOH como disolvente de extracción a 110ºC durante 5 minutos.

En cuanto a la validación del método, este mostró buena linealidad, exactitud (recuperaciones del 90%) y precisión (RSD< 10%). Tanto los LODs como los LOQs obtenidos estuvieron muy por debajo de los requeridos por la legislación europea. Por último, se aplicó a 20 muestras, destacando la presencia de algunos compuestos cuya presencia en la formulación de estos productos estará prohibida desde el 30 de julio de 2015.

CAPÍTULO II. DETERMINACIÓN DE FUNGICIDAS EN VINO Y SUBPRODUCTOS DE VINIFICACIÓN

2.1. Análisis rápido de fungicidas en vinos blancos del Noroeste de España mediante microextracción-emulsificación asistida por ultrasonidos y cromatografía de gases-espectrometría de masas.

Para determinar fungicidas en vinos blancos se propuso como técnica de extracción la microextracción-emulsificación asistida por ultrasonidos (USAEME). Esta técnica, desarrollada por el

grupo de investigación en el que se llevó a cabo esta Tesis, emplea volúmenes mínimos de disolvente orgánico (µL), por lo que se considera una técnica respetuosa con el medioambiente en comparación con técnicas clásicas de extracción líquido-líquido (LLE). Además es una técnica rápida y muy sencilla.

Se estudió la influencia del disolvente, el pH de la muestra, la adición de sal, así como el tiempo y temperatura de extracción para obtener las condiciones más eficaces. Finalmente se emplearon 200 µL de cloroformo a pH=3 (el propio de la muestra), añadiendo un 10% de sal y realizando las extracciones durante 5 minutos a temperatura ambiente.

El método propuesto mostró recuperaciones entre 70-115% con RSD< 12%, obteniéndose LODs a niveles de sub-ng mL^{-1}.

Por último se aplicó al análisis de vinos blancos de 5 Denominaciones de Origen gallegas, en los cuales se detectaron entre 2-7 fungicidas por muestra, llegando hasta concentraciones totales en algunos casos de 700 ng mL^{-1}.

2.2. Determinación de fungicidas en bagazo de uva blanca mediante extracción con líquidos presurizados y cromatografía de gases-espectrometría de masas en tándem.

Este es el primer trabajo documentado en el que se desarrolla un método analítico para determinar simultáneamente diferentes tipos de fungicidas en uno de los principales sub-productos de vinificación como es el bagazo. Como técnica de extracción se ha empleado PLE y los principales parámetros que afectan a su eficacia se optimizaron mediante diseños experimentales. De esta forma, los analitos de interés se extrajeron con una mezcla de hexano:acetona, a 120ºC durante 5 minutos.

Para la determinación se empleó tanto GC-MS como GC-MS/MS, ya que el uso de MS en tándem permite mejorar la selectividad y la sensibilidad analítica al minimizar las interferencias de la matriz. De esta forma, ambas técnicas de análisis fueron comparadas obteniéndose IDLs y LODs hasta dos órdenes de magnitud menores empleando GC-MS/MS.

Esto supone un gran avance para la determinación de niveles traza de estos compuestos en muestras reales. El método propuesto también presentó recuperaciones cuantitativas, con RSD< 12%.

Finalmente el método se pudo aplicar con éxito al análisis de 18 muestras de bagazo de uva blanca de varias Denominaciones de Origen gallegas, detectándose fungicidas en todas ellas y destacando la presencia de tebuconazole y dimethomorph en el 94 y 87% de las muestra analizadas, respectivamente.

Aunque la concentración de fungicidas en bagazo no se encuentra regulada, cabe desatcar que la concentración de una de las muestras analizadas se encuentra por encima del límite establecido para uvas de mesa.

CAPÍTULO III. DETERMNACIÓN DE HIDROCARBUROS AROMÁTICOS POLICÍCLICOS EN SUPERFICIES DE JUEGO Y ACEITE DE OLIVA

3.1. Investigación de PAHs y la presencia de otros contaminantes peligrosos en superficies de caucho de neumáticos reciclados. Caso de estudio: parque infantil interior en restaurante de un centro comercial.

El objetivo de este estudio era determinar la presencia de PAHs en una superficie de juego infantil de caucho reciclado situada en el interior de un restaurante y demostrar la transferencia de estos contaminantes prioritarios y de otros como plastificantes y antioxidantes al aire y al agua que se encuentran en contacto con la misma.

Para el análisis de la propia superficie se empleó la extracción asistida por ultrasonidos (UAE), una técnica rápida y muy sencilla, seguida de GC-MS. De esta forma 14 de los 16 PAHs estudiados se detectaron en la superficie, con concentraciones totales de 170 μg g^{-1}. Otras sustancias como plastificantes y antioxidantes pudieron ser también detectadas en estas muestras, destacando los elevados niveles del ftalato DEHP (> 3000 μg g^{-1}). De esta forma se demuestra que aunque la legislación española considera los neumáticos fuera de uso, a partir de los que se fabrican estas superficies, como residuos no peligrosos, estos contienen numerosas sustancias contaminantes de primer orden en concentraciones, en algunos casos, muy elevadas.

El análisis del aire y agua puestos en contacto con la muestra, se llevó a cabo mediante HS-SPME seguida de GC-MS. Esta técnica fue previamente optimizada por el grupo de investigación en el que se realizó esta Tesis. Con este técnica, 9 de los 16 PAHs fueron encontrados en el aire, incluyendo uno de los más tóxicos, el B(a)A. En el agua que estuvo en contacto con la superficie de juego infantil se detectaron 9 PAHs, con una concentración total de 2223 ng mL^{-1}, destacando nuevamente la presencia de B(a)A (681 ng mL^{-1}).

Este estudio demuestra la presencia de PAHs en el aire que se encuentra sobre las superficies de juego infantiles, por lo que estos compuestos podrían ser fácilmente inhalados, lo que supondría un riesgo para los niños. Asímismo, se demuestra una transferencia de los contaminantes a las aguas de lavado de las superficies (concentraciones de ppm), por lo que estos compuestos pueden ser fácilmente incorporados a las aguas de alcantarillado.

3.2. Optimización de las condiciones experimentales para el análisis de hidrocarburos aromáticos policíclicos en aceite de oliva mediante microextracción en fase sólida con vacío.

Por último, en este apartado se explican brevemente los resultados obtenidos durante la estancia en la *Technical University of Crete*, para la determinación de PAHs en aceite de oliva.

El objetivo era emplear la microextracción en fase sólida con vacío (Vac-SPME) para realizar la extracción de los 5 PAHs más volátiles. Para obtener las condiciones más favorables se estudiaron los parámetros más críticos que podían afectar a la eficacia de extracción: tipo de fibra, tiempo de evacuación del aire antes de la introducción de la muestra, tiempo de equilibrio una vez que la muestra se encuentra en el vial y tiempo y temperatura de extracción.

Las condiciones más favorables se obtuvieron empleando PDMS como recubrimiento de la fibra, realizando vacío durante 30 segundos, con un tiempo de equilibrio de 10 minutos y exponiendo la fibra al espacio de cabeza durante 30 minutos a 25ºC.

CONCLUSIONES GENERALES

A lo largo de los trabajos multidisciplinares que se engloban en esta Tesis Doctoral, se desarrollaron nuevas técnicas de preparación de muestra seguidas de GC-MS y en algunos casos GC-MS/MS para determinar productos de cuidado personal, así como contaminantes de interés prioritario en un amplio rango de matrices (vino, bagazo, superficies de caucho reciclado o aceite de oliva). La mayoría de los compuestos analizados se encuentran regulados por la legislación europea y muchos de ellos están siendo prohibidos o restringidos para su uso.

Las técnicas de preparación de muestra empleadas son rápidas, sencillas y de fácil implementación en cualquier laboratorio. Además, implican mínimos consumos de disolventes orgánicos y de muestra, lo que abarata los costes. Las técnicas empleadas fueron: µ-MSPD y PLE para el análisis de productos de cuidado personal, USAEME y PLE para el análisis de vinos y sub-productos de vinificación, UAE y SPME para el análisis de superficies de juego de caucho reciclado y Vac-SPME para la determinación de aceite de oliva. La optimización de las variables que afectan a los distintos procesos de extracción se llevó a cabo mediante diferentes tipos de diseños experimentales, lo que permitió minimizar el número de experimentos y conseguir, a la vez, una interpretación más sencilla y completa de los resultados, al considerar las interacciones entre los factores estudiados.

La evaluación de los parámetros de calidad de los métodos propuestos en términos de linealidad, repetibilidad, reproducibilidad, exactitud y precisión proporcionó resultados satisfactorios, con LODs muy por debajo de los exigidos por la legislación aplicable en cada caso. En ese sentido, el empleo de MS/MS permitió la disminución de estos límites obteniendo una alta selectividad y sensibilidad analítica.

En resumen, los trabajos presentados en esta Tesis Doctoral suponen una gran contribución al desarrollo y validación de nueva metodología analítica basada en técnicas de microextracción y análisis cromatográfico para el análisis de productos de cuidado personal, así como de contaminantes de interés prioritario en otras matrices.

MAIN CONCLUSIONS

During the development of the research works included in this PhD Thesis, new sample preparation procedures followed by GC-MS and GC-MS/MS were developed to analyze personal care products (PCPs) and priority contaminants in several matrices (wine, bagasse, tyre rubber surfaces, or olive oil). Most of the studied chemicals are regulated according European Legislation and some of them are restricted or banned for their use.

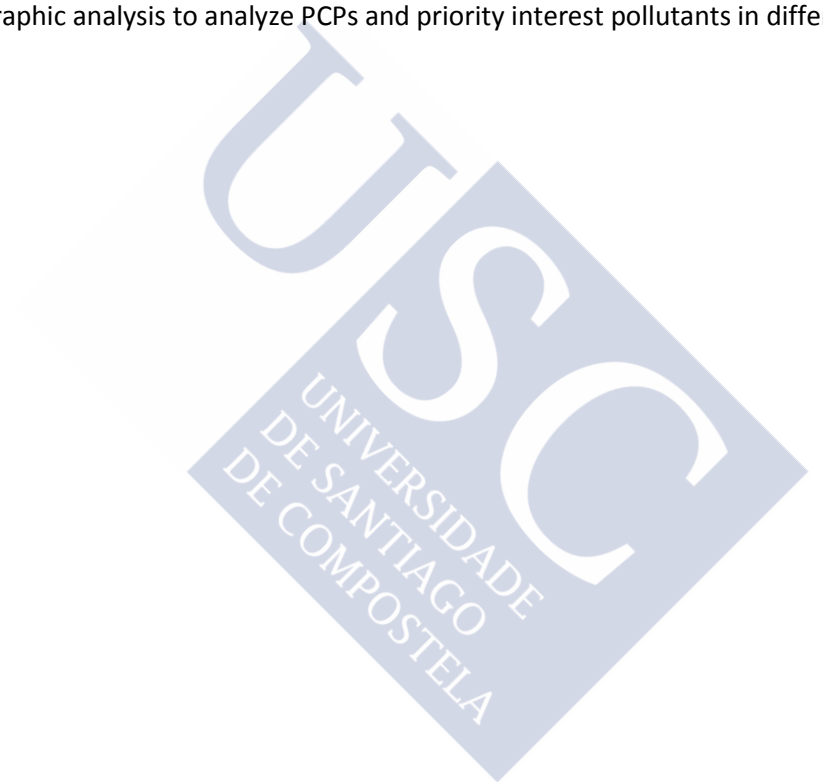
Developed sample preparation techniques were fast and simple, and easy to implement in laboratories. They also employed a minimum consumption of organic solvents, and sample amount in order to reduce costs, residues, and risks. The techniques employed were: µ-MSPD and PLE for the

analysis of PCPs, USAEME and PLE to analyze wine and vinification by-products, UAE and SPME to analyze rubber recycled tyre playground surfaces and Vac-SPME for the determination of olive oil.

Optimization of the extraction variables was carried out by means of experimental designs, in order to minimize the number of experiments and to achieve an easy interpretation of the results, since interactions between studied factors are considered.

Methods quality parameters were evaluated in terms of linearity, repeatability, reproducibility, accuracy, and precision showed satisfactory results, and the LODs were well below than the legislation limits. In this sense, the use of MS/MS allowed a decrease of the obtained limits providing high selectivity and analytical sensitive.

In summary, the research developed during this PhD Thesis, makeup a large contribution to the development and validation of new analytical methods based on micro-extraction techniques and chromatographic analysis to analyze PCPs and priority interest pollutants in different matrices.



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Durante el desarrollo de esta Tesis Doctoral se han publicado o están pendientes de publicación los siguientes trabajos:

- Maria Llompart, **Maria Celeiro**, J.Pablo Lamas, Lucia Sanchez-Prado, Marta Lores, Carmen Garcia-Jares, *Analysis of plasticizers and synthetic musks in cosmetic and personal care products by matrix solid-phase dispersion gas chromatography-mass spectrometry*, J. Chromatogr. A, 1293 (2013) 10-19. doi: 10.1016/j.chroma.2013.03.067

- Maria Celeiro, Eugenia Guerra, J.Pablo Lamas, Marta Lores, Carmen Garcia-Jares, Maria LLompart, Development of a multianalyte method based on micro-matrix-solid-phase dispersion for the analysis of fragrance allergens and preservatives in personal care products, J. Chromatogr. A, 1344 (2014) 1-14. doi: 10.1016/j.chroma.2014.03.070

- **Maria Celeiro**, J. Pablo Lamas, Maria Llompart, Carmen Garcia-Jares, *In-vial micro-matrix-solid phase dispersion for the analysis of fragrance allergens, preservatives, plasticizers, and musks in cosmetics*, Cosmetics 1 (2014) 171-201. doi: 10.3390/cosmetics1030171

- **Maria Celeiro**, J. Pablo Lamas, Carmen Garcia-Jares, Maria Llompart, *Pressurized liquid extraction-gas chromatography-mass spectrometry analysis of fragrance allergens, musks, phthalates and preservatives in baby wipes*, J. Chromatogr. A, 1384 (2015) 9-21. doi: 10.1016/j.chroma.2015.01.049

- Maria Celeiro, Maria Llompart, J.Pablo Lamas, Marta Lores, Carmen Garcia-Jares, Thierry Dagnac, *Determination of fungicides in white grape bagasse by pressurized liquid extraction and gas chromatography tandem mass spectrometry*, J. Chromatogr. A, 1343 (2014) 18-25. doi: 10.1016/j.chroma.2014.03.057

- Carmen Garcia-Jares, **Maria Celeiro**, J.Pablo Lamas, Maria Iglesias, Marta Lores, Maria Llompart, *Rapid analysis of fungicides in white wines from Northwest Spain by ultrasound-assisted emulsification-microextraction and gas chromatography-mass spectrometry*, Anal. Methods, 6 (2014) 3108-3116. doi: 10.1039/C3AY42285B

- **Maria Celeiro**, J.Pablo Lamas, Carmen Garcia-Jares, Thierry Dagnac, Lourdes Ramos, Maria Llompart, *Investigation of PAH and other hazardous contaminant occurrence in recycled tyre rubber surfaces. Case-study: restaurant playground in an indoor shopping centre*. Int.J. Environ. Anal. Chem. 94 (2014) 1264-1271. doi: 10.1080/03067319.2014.930847

-Eugenia Guerra, **Maria Celeiro**, J.Pablo Lamas, Maria Llompart, Carmen Garcia-Jares, *Determination of dyes in cosmetic products by micro-matrix solid phase dispersion and LC-MS/MS*, J. Chromatogr. A., 2015 (in revision).